THE UNIVERSITY OF ALBERTA

PHYSICAL AND CHEMICAL STUDIES OF THE PROTEINS OF
RAPESEED (BRASSICA CAMPESTRIS VAR. TORO)

BY

© [illegible] CHAMNANWEJ

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA
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# PHYSICAL AND CHEMICAL STUDIES OF THE PROTEINS OF RAPESEED (*BRASSICA CAMPESTRIS* VAR. ECHO)

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The undersigned certify that they have read, and recommend to the faculty of Graduate Studies for acceptance, a thesis entitled PHYSICAL AND CHEMICAL STUDIES OF PROTEINS OF RAPESEED (*Brassica campestris* var. Echo) submitted by Nuatong Chamnanwej in partial fulfilment of the requirements for the degree of Master of Science.

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# ABSTRACT

Proteins from hexane-defatted rapeseed meal (*Brassica campestris* var. Echo) were extracted with 0.01 M sodium pyrophosphate (pH 7.0), 10 percent (W/V) sodium chloride and 0.05 M acetic acid (pH 3.1) solutions. The extracts were purified by dialysis against suitable buffers. The proteins were separated into soluble and insoluble fractions during dialysis, as a part of the protein mixture was precipitated during this operation.

The lyophilyzed proteins from both the soluble and insoluble fractions after dialysis were subsequently dissolved in buffers and fractionated on Sephadex G-100 molecular sieve columns into their various components. The distribution of protein moieties of different molecular size ranges was examined and shown to consist of high, intermediate and low molecular weight proteins.

The complexity of each gel filtration fraction was then investigated on polyacrylamide gel columns, using tris-glycine buffer, pH 8.3, which revealed heterogeneity in the proteins extracted in the different solvent systems. The migration behavior of these protein moieties in the alkaline gels employed, suggested their neutral and basic character as well as the distribution of their molecular sizes. The 8 molar urea and dithiothreitol applied to the electrophoresis analysis appeared to demonstrate the dissociation and association phenomena of these proteins.

The amino acid analyses of each fraction were performed, and

the data collected support the identification of the different classes of proteins which was based on the molecular weight and solubility behavior. With respect to nutritional quality, different fractions of proteins extracted with the two neutral salt solutions and dilute acetic acid solution were generally comparable in the essential amino acid composition, with only minor variation in lysine, histidine and arginine.

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# I. INTRODUCTION

In solving the problem of providing adequate protein for an expanding world population, any large-scale increase in our protein requirement must be met by developing protein concentrates from plant sources. Therefore, more research on the nature and properties of plant proteins is needed. It has been shown that oilseeds can be a source of protein of good nutritive value and examples have been given of the ways in which non-traditional processes can lead to a fuller exploitation of oilseeds as a source of protein for human consumption (Kaye *et al*. 1961; Milner, 1966; Parpia and Subramanian, 1966; Dimler, 1969).

Rapeseed protein is of current interest since rapeseed is now an economic crop in Western Canada, primarily as a source of vegetable oil. Production of 37.1 million bushels of rapeseed in 1969 was almost triple the 1964 production of 13.2 million bushels. Rapeseed, crushed in modern mills, yields approximately 40 percent oil and 50 percent meal; the remainder is moisture. The meals left after oil extraction contain large quantities of protein and are used primarily as animal feeds, although in some countries, they are used as a fertilizer. Two species of rape are grown in Canada: *Brassica napus* L., commonly called Argentine rapeseed and *Brassica campestris* L., commonly called Polish rapeseed. The species *B. campestris* L. is the type most commonly grown in Western Canada, probably it is more

resistant to frost in the seedling stage than Argentine and may also be seeded late enough to escape the frost damage. The early maturity of the Polish is also a distinct advantage though seeds are smaller than those of Argentine and yields of seed are lower.

With the development of new techniques and instruments, constituents of plant proteins have become more widely understood. Fractionation of proteins on Sephadex molecular sieve columns has resulted in much valuable information on protein from many sources. The Sephadex technique, which depends on differences in molecular size and shape, is useful because it gives a minimal alteration of the protein systems being separated. Another method, that of gel electrophoresis, applied to the study of plant proteins, has demonstrated the effectiveness of preliminary purication and revealed many previously unrecognized protein components in plant protein extracts.

Although extensive analyses have shown that rapeseed meal contains substantial amounts of sulfur containing and basic amino acids, few detailed investigations of rapeseed protein have been published. Some studies include results of analysis of the water, dilute aqueous ammonia, and salt soluble proteins (Finlayson, 1967; Bhatty *et al.* 1968). The present study was undertaken to obtain a better knowledge of the protein of rapeseed (*B. campestris* L. var. Echo) by (a) the study of its solubility in different extracting media, (b) fractionation according to molecular size, (c) determination of the complexity of its protein constituents, and (d) an examination of the amino acid content of each fraction.

# II. REVIEW OF LITERATURE

## A. Nutritional Value of Oil Free Rapeseed Meal

Bell (1955) in his review on the nutritional value of rapeseed meal stated that the first experiments on this subject were published in Germany as long ago as 1872; whereas in Northern America reports first appeared in 1944, because it had been generally considered that rapeseed was less palatable and less readily digestible than many of the other commonly used high-protein meals of plant origin. At present, however, advancements in processing technology have resulted in better quality meals. A recent report on commercial solvent-extracted rapeseed meals made by Ballester *et al.* (1970a) suggested that the meals contain a high protein content with an adequate level of available lysine and that their amino acid patterns corresponded to a good quality protein.

Because of its use as protein supplement, more attention has been given to rapeseed meal protein than other nutrients in the meal. Wetter (1965) reporting the protein content of rapeseed determined by Downey (unpublished data), stated that Downey found that defatted ground seeds of *B. napus* gave mean values of 47.1 and 48.0 percent protein (N x 6.25); the mean values for *B. campestris* were 43.3 and 45.8 percent. Clandinin and Baylay (1963) showed that the protein content and the amino acids of the protein

differed among varieties and strains and also with the locality of growth. The latter influenced notably the lysine content of the protein, and to a smaller extent, the histidine, arginine, phenylalanine and leucine content. Lysine was significantly higher in B. campestris, while histidine was higher in one variety of B. napus. Sallans(1964) suggested that there was a reverse correlation of protein and oil yield as related to environmental conditions; generally, low rainfall with resulting low soil moisture and higher temperature led to a lower yield of oil seeds with a higher protein to oil ratio.

Extensive denaturation of protein during the process of oil extraction (cooking the crushed seed prior to oil removal) has been observed by several workers. The damaging effect of heat upon amino acids was noted in the processing of various oilseeds, including soybean (Evan and Butts, 1948); sunflower (Renner, 1953); cottonseed (Conkerton, 1957); peanuts (Bensabat, 1958); mustard seed (Mustakas et al. 1962; McGhee, 1964); sesame (Carter, Cirino and Allen, 1961) and rapeseed (Clandinin et al. 1959; Clandinin and Tajcnar, 1961). The basic amino acids lysine, arginine and histidine, as well as cystine and tryptophan have been found to be affected. Among these lysine appeared to be the most sensitive and has been used as an index of destruction of the heat labile amino acids. McGhee et al. (1964) studying prolonged heating of mustard seed found lysine was reduced by 64.1, arginine by 30.1 and histidine by 15 percent. The damage loss is believed to be by destruction, or by a possible condensation with other amino acids in the protein chain in such fashion that the amino acids are not

liberated by digestion *in vivo* or by enzyme hydrolysis *in vitro*, but are liberated by acid hydrolysis; or a reaction with carbohydrates could occur, in which case amino acids would not be recovered upon acid hydrolysis (McGhee, 1964; Evan and Butts; 1948; Lea and Hannan, 1950).

Clandinin and Tajcnar (1961) studied the effect of cooking and conditioning temperatures on the lysine content of expeller processed meals and found that a decrease in the temperatures of both the cooker and conditioner resulted in an increase in lysine content in the meal. They also observed that lysine content of the meals correlated inversely with the final oil content. A marked reduction in the lysine content was found in the meal containing less than 6 percent residual oil. The average lysine content of the meals having over 6 percent residual oil was 4.8 percent of the protein. When the oil had been removed with a petroleum ether solvent (Clandinin and Baylay, 1963) and the resulting meal had not been heated, the average lysine content found for six varieties of rapeseed tested was 5.3 percent.

Clandinin, *et al*. (1959) found that the protein analyses of two expeller-processed commercial meals were 43.3 percent for *B. napus* and 33.9 percent for *B. campestris*. In the same report values were given for seed processed at different temperatures but no marked difference in the protein content was observed. Manns and Bowland (1963) determined the protein content of two solvent-processed meals of *B. campestris* and the values appeared to be 36.7 and 34.7 percent. These reports suggest, contrary to the above findings, that processing methods may affect the protein

content and careful control of the temperature during the process of oil extraction can avoid the protein damage of the meal.

The amino acid composition of rapeseed meal has been studied extensively, because of the nutritional importance of specific amino acids and the interest in characterizing the component amino acids as related to the physicochemical properties of rapeseed protein. Bell (1955) reviewed the data of Roche and Michel (1946), Agren, (1952) and an unpublished report by Wetter and McConnel on amino acid composition of rapeseed oilmeal. These data do not agree and furthermore are different from those reported by Goering *et al*. (1960). Miller *et al*. (1962), in a detailed study of the amino acid composition of the seed meals of 41 Cruciferae species, compared *B. napus* with *B. campestris* and found little difference in the amino acid content. They also found no difference between the varieties grown in Sweden and in Canada. Wetter (1965) compared amino acid content of various rapeseed meals and other protein supplements and showed that the greatest variation occurred in the lysine, arginine, and serine content. Rapeseed meal compares quite favorably with other vegetable protein concentrates according to recent reports of Finlayson (1965), Bell, Downey and Wetter (1967), and Ballester *et al*. (1970a). The results from these independent investigators were not comparable since differences in variety, meal sources, methods for oil removal, and amino acid assay techniques would affect the observed amino acid content.

Little research has been conducted on the carbohydrate content of rapeseed meal. Matét, Nentagne and Buchy (1949)

reported that the carbohydrate content of European rapeseed cake varied from 20 to 25 percent. These values are similar to those reported for linseed cake. Another more detailed investigation by Mizuno (1958) found that *B. napus* oil-free meal contained the following carbohydrates: fructose (0.51 percent), glucose (0.21 percent), sucrose (1.11 percent), raffinose (0.15 percent) and stachyose (0.19 percent). Trace amounts of a number of monosaccharides, namely, arabinose, galactose, ribose, xylose and rhamnose; and galacturonic acid were also reported by the same authors.

The oil content of rapeseed meal varies with method of oil removal, from 6 to 7 percent in expeller-processed meal, to between 1 and 4 percent in solvent extracted meal (Clandinin *et al.* 1959; Mustakas *et al.* 1965; Ballester *et al.* 1970a). Presumably, the fatty acid composition would be similar to the oil found in the original oilseed. Similar to other seed oils which belong to mustard family (Cruciferae), rapeseed oil contains large amounts of erucic acid, a $C_{22:1}$ acid, and gadolinic acid, a $C_{20:0}$ acid. distinguishing these oils from most other vegetable oils, which generally contain fatty acids with $C_{16}$ and $C_{18}$ chains. Fatty acid analyses of rapeseed oil obtained by gas chromatography (Craig, 1961; Stefansson and Hougen, 1964) have revealed a negative correlation between oleic and erucic acid contents and come variation in palmitic, linoleic, and eicosinoic acid contents, as well as extreme variability in erucic acid in two varieties of *B. napus*. A plant which produced inbred seed containing zero erucic acid was found in the variety Liho; and a plant that

produced 50 percent erucic acid in the oil was available from the variety Golden (Stefansson, Hougen and Downey, 1961). High contents of erucic acid have been observed to retard growth and produce other undesirable effects in animals given substantial quantities of rapeseed oil in their diet. However, these undesirable effects of rapeseed oil can be attributed also to deficiency of saturated acids (Beare et al. 1963). Attempts have been made to correct these above mentioned disadvantages of rapeseed oil by plant breeding and selection (Craig and Wetter, 1959; Downey and Harvey, 1963; Stefansson, Hougen and Downey, 1961; Stefansson and Hougen, 1964), however, further nutritional studies are required to predict accurately the ideal fatty acid composition.

No data on the vitamin content of rapeseed oilmeal had been published until 1956. In comparison with the meals from other vegetable sources such as soybean oilmeal and sunflower seed oilmeal, rapeseed meal appears to be richer in choline and niacin, similar in riboflavin and lower in pantothenic acid and thiamine (Klain et al. 1956). The calcium and phosphorus content of rapeseed meal is 0.6 and 1.0 percent respectively compared with 0.3 and 0.7 percent for typical soybean meal and 0.4 and 0.8 percent for linseed meal (Bell, Downey and Wetter, 1967). Ballester et al. (1970a) investigated the iron content of solvent-extracted rapeseed presscake meals from three processors and the average value of the meal was found to be 0.17 percent.

Rapeseed oilmeal is known to contain higher fibre content than other oilmeals. Klain et al. (1956) obtained values ranging

from 9 to 16 percent and found no difference in the fibre content between solvent extracted meal and expeller-processed meal. The crude fibre content of rapeseed meals was similar to that of sunflower seed oilmeal, but was about 2.5 times that of soybean oilmeal. This fact may have an important influence on the nutritive value of this meal. An average value 13.6 percent from industrial solvent extracted meals was found by Ballester *et al.* (1970a).

## B. Toxic Factors

The undesirable principles in rapeseed meal are mainly thioglucocides and related compounds. Although over 40 thioglucocides have been reported in plants, only three major ones have been identified in rapeseed species: gluconapin, glucobrassicanapin and progoitrin, which when hydrolysed by the enzyme myrosinase, give rise to 3-butenyl and 4-pentenyl isothiocyanates and 5-vinyl-2-oxazolidinethione, respectively. As to the present status of thioglucoside biosynthesis, it has been shown that certain amino acids are the precursors for these substances (Chisholm and Wetter, 1964; Underhill, Chisholm, and Wetter, 1962 and Underhill, 1965). The incorporation of the labeled precursors into the aglycones of progoitrin, gluconapin and glucobrassicanapin in maturing rape plants (*B. campestris* L. var. Arlo) suggested that the plant can synthesize amino acids by the condensation of acetate (as acetyl CoA) to $\alpha$-keto acid homologues of the original amino acid. These newly formed keto acids are then transaminated, and used in the synthesis of the

three major thioglucosides (Chisholm and Wetter, 1967).

Many reports (Kratzer _et_ _al_. 1954; Turner, 1948; Jackson, 1969) demonstrate that rapeseed meals cause a depression in growth and in many cases enlargement of the thyroid when fed to animals. L-5-vinyl-2-oxazolidinethione derived from a glucoside found in brassica seed was reported to produce enlargement of the thyroid and to impair proper functioning of this gland in chickens (Astwood _et_ _al_. 1949). The only isothiocyanates having significant antithyroid activity are those capable of cyclizing to form oxazolidinethione (Bell and Belzile, 1965). Growth inhibition has been studied in the mouse as an index of toxicity of rapeseed meal (Bell, 1957; Bell and Baker, 1957; Belzile, Bell and Wetter, 1963) and it has been shown that isothiocyanates and oxazolidinethione, enzymatically liberated from their parent glucosides, have about equal effects on growth rates in these animals; but it was observed that males were affected somewhat more severely than females.

As the amount of thioglucosides in meals from rapeseed limits their use as animal feed, investigations into varietal differences on content of the split products from thioglucosides were initiated by Wetter and Craig (1959). The isothiocyanate content of rapeseed grown in Western Canada was found to vary from 4.33 - 5.36 mg./g. oil-free meal, while the oxazolidinethione varied from 1.33 - 5.6 mg./g. of meal. Appelqvist (1962), in Sweden, attempted to learn if genetically controlled variation in content of these substances was great enough to form a basis for plant breeding and the results he obtained showed much greater

varietal deviation than those observed by Wetter and Craig (1959). The isothiocyanate showed variation from 2.5 - 9.2 mg./g. defatted meal and the amount of oxazolidinethione varied from 0.2 - 7.9 mg./g. meal. The variations found seem to indicate that further selection towards lower content of isothiocyanates and oxazolidinethiones is possible.

In 1969, Downey, Craig and Youngs reported that complete removal of sulfur containing toxic compounds may be possible through plant breeding. Partial success in eliminating these compounds has been achieved by developing varieties of turnip rape (B. campestris) which do not contain the glucosides of 4-pentenyl isothiocyanate and oxazolidinethione, and the isolation of B. napus varieties with very low levels of all three glucosides.

Josefsson (1970) observed the marked effect of sulfur fertilization on the total content of thioglucoside compounds. His investigation showed that the amount of glucosinolate was increased at a higher sulfate level and a reduction in glucosinolate content of rapeseed may be obtained by using fertilizers low in sulfur in sandy soils. The fertilization also affected the protein and amino acid content but the effect was less than that observed in the sulfur compounds.

A number of treatments have been proposed for detoxification of the meal. Acid hydrolysis, alcohol and aqueous extraction and steam processing showed little improvement (Bell, 1957). Goering et al. (1960) reported that enzyme treatment partially detoxified rapeseed meal; the toxic factors appeared to

concentrate in the soluble portion after enzyme treatment, and thus this fraction could be discarded. Since the release of the goitrogenic substances is mediated by enzymes already present in the meal, the meal can be moistened to enzymatically liberate the isothiocyanate, which then may be removed by steam distillation (Mustakas et al. 1965). In present commercial methods, crushed rapeseed is cooked for 30 min. without the addition of water at a temperature of 80-91°C to inactivate the myrosinase. This operation leaves the bulk of thioglucoside in the meal. Though it has been known that intact thioglucosides are harmless (Belzile et al. 1963), they can be hydrolyzed in the digestive tract by the introduction of myrosinase from other foods or by enzymes produced by certain intestinal bacteria and then become toxic (Bell and Belzile, 1965). The thioglucosides are observed to be decomposed by heating with salts of iron, copper or nickel (Sallans et al. 1967). Autoclaving or steam stripping was reported to result in the disappearance of about 90 percent of the oxazolidinethione (Bell and Belzile, 1965). Recently, Eapen, Tape and Sims (1968, 1969) reported that a white, bland, defatted, thioglucoside-free flour can be prepared from rapeseed. Wet heat treatment of the meal prior to water extraction was found to be superior to dry heat treatment and it also facilitated the removal of the fibrous hull resulting in a more attractive meal. Similar success was reported by Ballester et al. (1970b) who made a comparison of direct and indirect steaming and water extraction under a variety of conditions, for detoxification. The best result was obtained with double water extraction, first extraction of 14 hrs. being

followed by a second extraction for 1 hr. at room temperature. This treatment can reduce 84 percent of oxazolidinethione, the factor directly responsible for toxicity, and 77 percent of isothiocyanates. Net protein utilization was increased to a level corresponding to that of animal proteins, from 40 to 60 percent.

## C. Solubility of Seed Proteins

### (1) Classification based on solubility (Osborne, 1919; West and Todd, 1963)

Solubility formed the original basis of distinction for several kinds of proteins, and methods based on this property are still very useful in both the analysis and fractionation of protein mixtures. Different species of seeds may contain different amount and types of proteins as judged by solubility criteria. In general it is found that some proteins are soluble in water, some are soluble in saline solutions and others are insoluble in either of these solutions but are soluble in solutions of acids or alkalies. In the seeds of cereals 70 to 90 percent of the protein may be soluble in alcohol. According to their solubility behavior in such solvents, the proteins which normally occur in plants can be characterized as follows: albumins, soluble in water or dilute salt solutions and precipitated from solution by saturation with ammonium sulphate; globulins, soluble in salt solutions but not, or only slightly, soluble in water and precipitated from solution by half saturation with ammonium sulfate; protamins, soluble in 70 to 80 percent ethanol but not in absolute ethanol, neutral

solvents, or in water; glutelins, soluble in dilute acids or alkalies but not in water, salt solution or aqueous alcohol; and histones, soluble in water, dilute acids and alkalies, but not soluble in dilute ammonia. Extraction are therefore generally performed by using a series of solvents, chosen to successively dissolve the main classes of proteins expected to be present in the seed.

(2) Factors which affect solubility

According to Sober _et al_. (1965) the following five factors are important:

a. pH

The solubilities of most proteins are minimal in the vicinity of their isoelectric points. The change may be quite sharp, with solubility increasing by as much as a factor of 10 when the pH is changed one unit in either direction. pH therefore is a factor of prime importance and a procedure such as salting out which produces good separation at one pH may give no separation at another, or the order of precipitation may even be reversed.

b. Temperature

The effect of temperature on the solubility of any particular protein is quite unpredictable. It is useful to be aware, however, that the difference in solubility between 0° and 25°C may be as much as an order of magnitude in the other direction. For a fractionation

to be reproducible, the temperature must be specified.

c. Ionic strength

At very low ionic strength, the solubility of proteins in general increases with increasing salt ("salting in"). As ionic strength is increased, however, a maximum is reached beyond which solubility decreases continuously ("salting out"). While sensitivity to small differences is generally greater at low ionic strength, a great many proteins are fairly soluble even in pure water, so that the salting-in region is most often used in conjunction with other precipitating agents. However most proteins can be salted out within their pH stability range and are usually relatively insoluble in high salt concentrations. Salting-out, therefore, should always be considered as a likely first step in the fractionation of a complex mixture, particularly if a large scale operation is contemplated. Ammonium sulfate and sodium sulfate are widely used for this purpose.

d. Dielectric constant

Dipolar ions such as glycine increase the dielectric constant of water, while water-miscible organic solvents decrease it. It is always problematical whether these effects are of greater importance than more specific interactions in determining protein solubility in any given instance. Both types of agents have been used to advantage, and organic solvents (ethyl alcohol

and ethyl ether in particular) have been used as general precipitating agents in several total fractionation schemes. Such schemes require careful attention to variables such as pH, ionic strength, temperature, and protein concentration at each step. The lability of proteins in the presence of organic solvents generally requires that the temperature be kept close to the freezing point of the solution throughout.

e. Other ions

Certain metal cations (Hg, Cu, Zn, Ba) as well as a variety of simple and polymeric anions (sulfate, trichloracetate, tungstate, heparin, polyglucose sulfate, etc.) are capable of forming slightly soluble salts with proteins, under the right conditions of pH and salt concentration. These and many more have been used in special situations to facilitate the separation of proteins.

## D. Characterization of Oilseed Proteins

### (1) Oilseed proteins other than rapeseed

It has been generally accepted that the major globulins of dicotyledons and the prolamines and glutelins of monocotyledons are reserve proteins, i.e. they are digested enzymatically on germination, whereupon amino acids and peptides are made available for protein synthesis (Danielson, 1956). The two major groups of globulins mentioned most often in the literature in peanut proteins are arachin and

chonarachin. The former, which accounts for about two-thirds of the protein, is precipitated at 40 percent saturation with ammonium sulfate from a salt extract of the peanut while the latter remains in solution (Johns and Jones, 1916; Brohut and Sadegren, 1954). Arachin forms a reversibly associating system in solution (Johnson and Shooter, 1950) depending on pH, salt concentration and type of salt. Lowering the pH and increasing the salt concentration promotes association. High concentrations of sulfate ions are especially effective in promoting almost complete association, but even in small amounts they appear to prevent dissociation. Changes in the conarachin fraction during germination were observed by purification of these proteins by column chromatography on diethylaminoethyl (DEAE) cellulose (Dechary _et_ _al_. 1961). These fractions were studied further by Evans _et_ _al_. (1962) who used polyacrylamide gel electrophoresis and demonstrated the complexity of composition of the crude fractions.

$\alpha$-Conarachin,which is homogeneous by gradient elution chromatography on DEAE cellulose, was shown to contain several closely related components on electrophoresis. Sedimentation analysis of the peanut globulins confirmed that they are part of an associating system. Dieckert _et_ _al_. (1962) studied the composition of subcellular fractions of protein by homogenization and differential centrifugation from nonaqueous media. Two protein rich fractions, one of which appears to be from aleurone cells, were obtained. The proteins in both fractions appear similar as judged by

chromatography and electrophoresis. Tombs (1965) fractionated and analyzed peanut proteins by DEAE-Sephadex chromatography and acrylamide gel electrophoresis. A simple method of preparing arachin by means of precipitation with calcium salts resulted in a very pure product. He also observed evidence of two forms of arachin which he attributed to polymorphism.

Considerable progress has been made in the purification and characterization of soybean protein. About 90 percent of the soybean proteins are globulins or glycinin (Osborn and Campbell, 1898) obtained by salt extraction, ammonium sulfate precipitation and dialysis. Ultracentrifuge studies of unfractionated soybean protein at pH 7.6 - 7.8, 0.5 ionic strength, indicates 4 well resolved components having approximate sedimentation constants of 2, 7, 11 and 15 S (Naismith, 1955 and Wolf and Briggs, 1956). The 11 S fraction is present to the greatest extent; the 11 S and 7 S component together account for 70 percent of protein (Wolf and Smith, 1961). When a concentrated solution of the aqueous extract of meal is cooled, a cold-insoluble fraction is obtained, which is primarily 11 S protein (Briggs and Wolf, 1957 and Naismith, 1955).

In extracts from defatted soybean meal, 7 S and 11 S globulins predominate in protein bodies (Wolf, 1970). Both the 7 S and 11 S components observed in acid-precipitated soybean protein or glycinin undergo similar reactions. At least three are known: a) dissociation into subunits (Wolf

and Briggs, 1956); b) association into noncovalent-linked polymers (Naismith, 1955) and c) dissociation of disulfide-linked polymers (Briggs and Wolf, 1957). At pH values below the isoelectric point, decreasing pH favors dissociation and higher salt concentration favors association. Sulfate ions cause a marked shift in association leading to formation of large amounts of unresolved high molecular weight material (Rachis et al., 1957). In addition, the tendency of low temperatures and specific ions to promote aggregation of the soybean globulin has been noted (Wolf and Sly, 1967). The so-called cold-insoluble fraction, 11 S, was 90 percent purified as judged by ultracentrifugation. Particle weights of 363,000 by sedimentation diffusion and 345,000 by light scattering were compared to 380,000 for the material obtained by ammonium sulfate fractionation (Wolf, Babcock and Smith, 1962). The differences in solubilities of the four components, 2 S, 7 S, 11 S and 15 S, with changes in ionic strength, provided a method of purification of the 11 S fraction as described by Eldridge and Wolf (1967). Fractionation precipitation gave a yield of purified 11 S protein contaminated with only about 7 percent of a component sedimenting in the 7 S region. However, there is also possibility of partial dissociation of 11 S into 7 S monomers. Extensive dissociation of the purified 11 S into 2 S and 7 S components in 0.03 ionic strength tris-citrate buffer, pH 8.6 has been observed. Similar results were obtained when Roberts and Briggs (1965) tried to purify the

7 S component by gel filtration on Sephadex G-100 and found it to contain a trace amount of 11 S. This component will associate to yield a 9 S form when electrolyte concentration is lowered from 0.5 to 0.1$\mu$. The molecular weight of the 9 S component was found to be about 650,000 and the 7 S, 330,000. The characteristic 7 S$\longrightarrow$9 S dimerization was also confirmed by the recent report by Koshiyama (1968a). The molecular weight of the 7 S protein of soybean globulins was found to be about 180,000 to 210,000 by different methods described by the same author (Koshiyama, 1968b).

Several papers have dealt with the amino acid composition of soybean proteins. Van Etten et al. (1959) reported the amino acid composition of soluble, acid precipitated, and heat coagulated protein fractions. Krober and Gibbon (1962) have estimated nitrogen in soybeans and estimated amino acids by ion exchange chromatography on soybean hulls, dehulled soybean meal, isolated protein, and the residue; as well as on whey proteins, on the hypocotyl, and acid-precipitated proteins of the hypocotyl. Over 30 percent of the nitrogen in immature soybeans was nonprotein nitrogen compared to 4 or 5 percent in the mature seed.

Studies on the nitrogenous constituents at various stages of development of coconut (Baptist, 1963) suggested that amino acids originate in the sap and are drawn selectively for the synthesis of the liquid endosperm protein. The presence of certain amino acids in the liquid endosperm before the endosperm has commenced to form, suggests

that for the synthesis of the endosperm protein, the soluble nitrogen may be drawn from these amino acids in the initial stages. Once cellular organization and differentiation have begun, it was shown that the solid endosperm contains all amino acids found in the corresponding liquid endosperm as well as others which the cells produce for their own requirements and are not detectable in the liquid endosperm. Small amounts of all the amino acids present in the newly formed cells will then diffuse out slowly into the liquid endosperm, so that both processes are taking place simultaneously. Sedimentation analyses (Sjogren and Spychalski, 1930) have shown that solutions of coconut globulin, cocosin, extracted with 10 percent sodium chloride, contain two components: the main one with molecular weight about 208,000 and a minor component of weight about 104,000. The concentration of the minor component increases in alkaline solutions. Such dissociations have been observed for other seed proteins, especially for the peanut and soybean proteins already described. The extractability of protein (nitrogen) from expeller and solvent extracted poonac (the press-cake left after extraction of oil from dried coconut kernel) with dilute aqueous HCl, NaOH, and salt solutions has been investigated by Chelliah and Baptist (1969), the protein of the former being more soluble. Under optimum conditions, approximately 40 percent and 55 percent of the poonac protein nitrogen was extractable with 0.15 percent aqueous acid and alkali respectively. Dilute salt solutions were found to

have a comparatively poor solubilizing effect and increasing the fat content of expeller poonac to 10 percent increased the nitrogen extracted by acid but not by alkali solutions. The amino acid content of coconut press-cake presented in the same paper suggested the limiting amino acids are lysine, methionine and tryptophan. The efficiency of protein extraction from coconut flour (Chandrasekaran and King, 1967) can be significantly increased from 49 to 85 percent by treatment with fungal enzymes prior to extraction. Postalotiopsis westerdijkii, a cellulolytic mould, produced a freely diffusing, extracellular enzyme capable of solubilizing hydrocellulose and amorphous cellulose when grown on coconut flour-solka-floc (2:1) medium at 31° for 11 days.

The most limiting amino acid in coconut milk preparations is lysine, followed by threonine, sulfur amino acids and isoleucine. Animal feeding experiments did not show any significant difference between the protein qualities of the control and enzyme-treated coconut milk preparation.

The proteins in cottonseed meal were fractionated by using classical fractionation procedures (Jone and Csonka, 1925) to yield six fractions. The $\alpha$- and $\beta$- globulins, glutelin, and pentose protein composed the major fractions, while protamine types were absent. Spies et al. (1941) found the relatively minor water-soluble fraction to consist of a series of low molecular weight proteins varying widely in polysaccharide content. Martinez and Frampton (1962) using ethanol solutions of varying salt content isolated

three protein fractions from cottonseed. Each fraction contained non-reducing carbohydrate and gave a single peak in the ultracentrifuge with an estimated $S_{20,w}$ value of 2, but showed many components when analyzed with cation exchange cellulose chromatography. Amino acid analyses showed the fractions to differ from each other and from whole meal. Rossi-Fanelli _et_ _al_. (1965) isolated a major fraction of cottonseed protein which they called acalin A. It is a cold-insoluble fraction, which dissolves in salt solutions at ionic strength greater than 0.2, and is precipitated by 70 percent saturated ammonium sulphate. This protein behaved as a homogeneous monodisperse system by ultracentrifugation, cellulose acetate electrophoresis and chromatography on DEAE-Sephadex and has a sedimentation value of 9.2 S. Conkerton and Frampton (1959) reported that the number of $\epsilon$-amino groups of lysine in several proteins, which are free to react with 2, 4-dinitrofluorobenzene, was reduced when the proteins were exposed to reaction with gossypol. A study of the relative importance of gossypol and raffinose in the binding and destruction of lysine and in impairing the nutritive value of cottonseed has shown that raffinose reduces lysine content when heat is applied; 1 percent gossypol and 10 percent raffinose are comparable in reducing the level of free $\epsilon$-amino lysine in cottonseed protein; and the nutritive index is correlated with the free $\epsilon$-amino groups of lysine of the protein (Martinez and Frampton, 1961).

The proteins of sunflower seeds and oilfree meal were

extracted with 10 percent sodium chloride and then precipitated by adding ammonium sulphate to 80 percent saturation (Joubert, 1955). Of the four components apparent in the ultracentrifuge, the main component had a sedimentation content of 11.7 S and it was obtained in practically pure form by precipitation of the extract with 20 - 30 percent saturated ammonium sulphate. The molecular weight, determined by sedimentation and diffusion experiments, is 343,000. One component with $S_{20} = 1.67$ S is precipitated in the range of 40 to 80 percent saturated salt. It is polydisperse and has a molecular weight of about 19,000.

(2) Rapeseed Proteins

a. Protein synthesis:

Incorporation of $^{14}C$ into rapeseed plants (B. napus L.) suggested that the synthesis of their reserve proteins proceeds over a substantial period of seed growth. Two $^{14}C$ labelled proteins were isolated from a water extract of the oil-free seed meal. The specific activities of the two proteins and of some amino acids suggested rate differences in protein synthesis, or alternatively, conversion of one protein into another. There was a point for maximum $^{14}C$ incorporation which was different for the two proteins (Finlayson, 1966). An extensive study was made by the same investigator (Finlayson, 1967) on rapeseed of the same species (B. napus). Changes in the amino acid composition have been

observed from the time that the seed contains a small amount of protein nitrogen until it is mature. The finding that there were no other amino acids appearing in the N-terminal position during the growing period, except to increase the amounts of those already present, and also that there was a steady decrease in N-terminal amino acids relative to the total amount of protein, suggested that synthesis of protein proceeds from the N-terminus to the C-terminus of protein chain. The results of these analyses ruled out the possibility that the storage protein is produced by the condensation of similar polypeptide subunits and the data did confirm the previous $^{14}C$-labelling experiments, in that the protein synthesis occurred over the entire period examined in the plant's growth.

### b. Comparative studies of seed proteins of Brassica species in relation to taxonomy.

Many recent papers (Vaughan *et al*. 1966; Vaughan and Waite, 1967a, 1967b; Vaughan and Denford, 1968) have reported seed protein studies in relation to taxonomy of *Brassica* and *Sinapis* species. The protein analysis method employed in these investigations included serological gel diffusion, acrylamide gel electrophoresis, and starch gel electrophoresis techniques. A good correlation was demonstrated between the serological and the acrylamide gel methods of protein analysis (Vaughan

*et al.* 1966). The albumin and globulin protein components separated electrophoretically were identified by a comparison of the Rp values (the ratio of the distance of the fast-moving bands measured from the junction between the large and small pore gel to the middle of the bands). The results shown by electrophoretic methods agreed with established taxonomy even though the technique does not possess the obvious taxonomic advantage of antiserum absorption. Investigation on seed protein over a wide range of *Brassica* and *Sinapis* species by Vaughan and Denford in 1968 showed a variation from 2 to 4 globulin bands and 11 to 18 bands of albumin proteins. The greater number and spread of Rp values of albumin bands gave a better correlation with the established taxonomy than that shown in the legumes, where globulin proteins have taxonomic value only at the tribal and generic level (Boulter, Therman and Derbyshire, 1968; Jackson, Milton and Boulter, 1967).

### c. The proteins of rapeseed (*B. napus*) soluble in salt solutions.

The salt soluble proteins from rapeseed (*B. napus*) var. Nugget, have been extracted with 0.1 M sodium pyrophosphate, pH 7.0, and with 10 percent (W/V) sodium chloride and subsequently separated into a number of components on Sephadex G-75 and G-100, carboxymethyl cellulose and diethylaminoethyl cellulose columns (Bhatty, McKenzie and Finlayson, 1968). Two major

proteins, a neutral (the 12 S protein) and a basic one (the 1.7 S protein, M.W. 13,800 ± 300) account for 30 percent of the nitrogen in the extract and nine other minor components were found to be present in the pyrophosphate salt extract. The 10 percent sodium chloride extracts contained a water-insoluble protein (the 12 S protein) which constituted 21 percent of the nitrogen in the extract and appeared to be similar in properties to the 12 S protein obtained when the pyrophosphate salt solution was used as extracting agent. This protein, upon sedimentation analysis in 6 M urea solution and in 0.1 M glycine-HCl buffer (pH 2.2), appeared to be an aggregate of units of smaller molecular weight.

## E. Determination of Molecular Weight of Protein by Gel Filtration

Gel filtration is a form of liquid chromatography that has been used to fractionate a wide variety of substances. Numerous applications of gel filtration in the fractionation of seed proteins are exemplified by a few studies reported since 1967 (Eldridge and Wolf, 1967; Crow and Rothfus, 1968; Catsimpoolas and Ekenstam, 1969; Booth, 1970; Booth and Ewart, 1970). These authors used mainly cross-linked dextran gels (Sephadex), but other polymer gels such as those prepared from agar, starch, polyacrylamide, polystyrene, etc., are also capable of producing similar separations. Under standardized conditions, gel filtration has also been successfully applied in the determination of molecular weights of several proteins.

Lathe and Ruthven in 1956, using a water-swollen starch column, demonstrated that for a large number of solutes the elution volume varied with size of the solute. Although molecular weight is unlikely to be a good approximation for the size of protein molecules, due to uncertainty about the size and shape of macromolecules in solution, the correlation between molecular weights and gel behavior on dextran columns (Granath and Flodin, 1961) indicates that for a homogeneous series of macromolecules, size and molecular weight are closely related. Andrew (1962) obtained evidence that this was also true for a number of proteins on agar gel columns, and showed that gel-filtration can be used as a comparative method to give a useful estimation of the molecular weight of proteins. Many investigators (Whitaker, 1963; Granath and Flodin, 1961; Determann and Michel, 1966) have demonstrated that the elution volume of a protein from the gel is directly proportional to the logarithm of its molecular weight.

The experimental technique for the molecular weight determination is, in principle, the same as that for the separation of a mixture of substances. All that is required is the determination of the elution volumes. As a rule, the solvent volume which leaves the column between the application of the substance to the gel packing and its elution in maximum concentration is considered the elution volume. Since the molecular weight dependent elution volume of a given substance is easily reproduced on the same column, it is possible to predict the molecular weight of a studied substance and hence, the column must be calibrated for this purpose.

In order to make the calibration independent of the individual experimental conditions, many parameters have been explored for the best description of the elution behavior of substances. The $K_d$ value ($V_e = V_o + K_d \cdot V_i$; $V_e$ = elution volume, $V_i$ = solvent volume inside gel particles, $V_o$ = outer or void volume, $K_d$ = accessible fraction of the inner volume) (Wheaton and Baumann, 1953), initially introduced, does not seem to be very appropriate due to the uncertainties in the determination of the solvent volume imbibed by the gel particles. Whitaker (1963) used the ratio of the elution volume to the void volume, $V_e/V_o$, as a function of the logarithm of molecular weight, and a good linear relationship between molecular weights and elution volumes was obtained. Squire (1964) has explained the findings of Whitaker based on a model in which the elements of volume available to the solvent within the gel are approximated by a combination of cones, cylinders, and crevices assuming a globular structure for the proteins ($M \propto r^3$) and avoiding the $K_d$ value. Data from different authors do not agree very well with his equation. Laurent and Killander (1963) have introduced a concept of the volume fraction available ($K_{av} = V_e - V_o/V_t - V_o$) for a solute in the gel phase which appears to be most generally applicable to this purpose. The total volume of the gel phase, instead of the inner volume ($V_i$), is applied to its calculation. All the parameters involved can be determined accurately and the resulting $K_{av}$'s (elution constants) express the penetrability of a certain molecular size into a gel of defined porosity. There are many more concepts of such relationships some of which are empirical and some which were

derived on the basis of theoretical considerations of the dependence of the elution volume on the molecular weight. Most of these relationships have been applied repeatedly and found to be valid.

# III. MATERIALS AND METHODS

## A. Preparation of Rapeseed Protein

### (1) Oil extraction

The rapeseed (*Brassica campestris* var. Echo) employed for the preparation of protein were purchased from the Alberta Wheat Pool and stored in the cold room at 5°C throughout the experiments. The moisture content of seeds as received was found to be about 8 percent. The seeds were selected to be free from soil and weedseeds. To improve the protein extracts for further study, dry whole seeds were first crushed to a fine powder in a Quaker City Mill (Model 4-E) and the oil was extracted from a 3g. sample of freshly crushed seed in a Soxhlet apparatus with 150 ml. n-hexane (b.p. range 67-68°C) for 4 hr. The resulting material was allowed to dry in air overnight and stored at 5°C. About 40 percent oil was obtained.

### (2) Protein extraction:

Protein was extracted from the meal powder by suspending 3 g.of the pooled hexane-defatted meal in 60 ml. of the different extracting media, and shaking for 90 min. at 5°C. The extracting media employed were 0.05 M acetic acid (pH 3.1), 0.2 percent sodium hydroxide (pH 11.9) and two neutral salt solutions, 0.01 M sodium pyrophosphate and 10

percent sodium chloride. The extracts were centrifuged at 10,000 rpm for 20 min. in a Sorval refrigerated centrifuge. The supernatant fluid was removed by pipet and the residue re-extracted three times as before. The combined solutions of protein soluble in 0.01 M sodium pyrophosphate, 10 percent sodium chloride, 0.05 M acetic acid and 0.2 percent sodium hydroxide were dialyzed against 0.005 M borate buffer pH 8.6, distilled water, 0.005 M acetate buffer (pH 4.1), and 0.005 M borate buffer (pH 9) respectively at 5°C for 48 hr. A precipitate formed during dialysis in most cases, except when 0.2 percent sodium hydroxide was used as extracting agent. The precipitates were subsequently recovered after centrifugation and resuspended in 30 ml. of the original extracting solutions and dialyzed for 24 hr. The supernatants and the precipitates were separately lyophilyzed and the resulting protein yields were determined on a dry weight basis using the Folin-Ciocalteau reagent.

### B. Fractionation of Protein Extracts on Sephadex G-100

The freeze-dried extract (varying from 100 - 250 mg.) was dissolved in a small volume of appropriate buffer and centrifuged clear at 7000 rpm. The sample was chromatographed on a Sephadex G-100 column (2.5 x 45 cm.) at 5°C using the dissolving buffers as eluants. Three and 4 ml. fractions were collected at a flow rate of 30 ml./hr. Protein in the column effluents was detected spectrophotometrically by measuring the absorbance at 280 mμ. Absorbance vs. tube number was plotted graphically. The effluent

in the tubes corresponding to each peak were pooled and dialyzed separately in the cold (5°C) for 24 hr. by continuous stirring against three changes of buffer. The experiments for all samples were carried out in a similar fashion and the experimental conditions were as given in Table 1.

## C. Polyacrylamide Disc Gel Electrophoresis

Polyacrylamide disc gel electrophoresis was performed essentially as described by Davis (1964). The gel was composed of two layers, with 8 M urea in both 2.5 percent stacking gel (pH 6.6 - 6.8) and 7.5 percent separating gel (pH 9.5). Stacking gel, 0.18 ml. was carefully layered over 0.2 ml. of 40 percent sucrose in vertical tubes of 7.0 cm. in length and 0.5 cm. in inner diameter, and polymerized by exposure to light for 20 min. After polymerization of the stacking gel, the tubes were filled with separating gel solution and the gel allowed to polymerize in the dark for 30 min. The 40 percent sucrose was then removed and the tubes were placed in the electrophoretic reservoir with the large pore layer upwards. The sample, 20 mg., was dissolved in 1.25 ml. of the solvent consisting of 8 M urea-0.001 M dithiothreitol (DTT) and about 100 $\mu$l of the sample solutions was applied per tube. Electrophoresis was conducted at 5°C for an hour at 2.0 mA per tube for 20 min. and then increased to 4.0 mA per tube for the remaining 40 min. After completion of the electrophoresis, the gels were immersed in 12.5 percent (W/V) trichloroacetic acid (TCA) for 20 min. to fix the protein in the gel, and then stained with a freshly prepared 1:20 dilution of 1 percent aqueous stock

Table 1.

Experimental Conditions for Chromatography on Sephadex G-100.

| Samples | Column Size | Flow Rate ml./hr. | Size of Fraction ml./tube | Eluant |
|---|---|---|---|---|
| 7 ml. containing 250 mg. protein extracted with 0.01 M sodium pyrophosphate (pH 7), soluble during dialysis. | 2.5 x 45 cm. | 30 | 3 | 0.005 M borate buffer, pH 8.6 |
| 6 ml. containing 150 mg. protein extracted with 0.01 M sodium pyrophosphate (pH 7), insoluble during dialysis. | 2.5 x 45 cm. | 30 | 3 | 0.005 M borate buffer, containing 0.005 M sodium pyrophosphate (pH 8.6) |
| 6 ml. containing 150 mg. protein extracted with 10 percent sodium chloride, water-soluble | 2.5 x 45 cm. | 30 | 4 | 0.005 M borate buffer, containing 5 percent sodium chloride (pH 8.6) |
| 6 ml. containing 150 mg. protein extracted with 10 percent sodium chloride, water-insoluble | 2.5 x 45 cm. | 30 | 4 | 0.005 M borate buffer, containing 5 percent sodium chloride (pH 8.6) |
| 5 ml. containing 120 mg. protein extracted with 0.05 M acetic acid (pH 3.1), soluble during dialysis | 2.5 x 45 cm. | 30 | 4 | 0.005 M acetate buffer, pH 4.1 |
| 4 ml. 100 mg. protein extracted with 0.05 M acetic acid (pH 3.1), insoluble during dialysis | 2.5 x 45 cm. | 30 | 4 | 0.005 M acetate buffer, pH 4.1 |
| 7 ml. 50 mg. protein extracted with 0.2 percent NaOH | 2.5 x 90 cm. | 15 | 4 | 0.005 M borate buffer, pH 9.0 |

solution of Coomassie Blue (COLAB) in 12.5 percent TCA. After 50 min. of staining the excess dye was removed by washing with 10 percent TCA and the gels were placed in fresh 10 percent TCA for viewing and storage. The migration of each protein band ($d_p$) and the tracking dye ($d_d$) from the boundary of large and small pore gels were measured at least three days after the gel had been prepared. The $R_p$ value was calculated by $R_p = \frac{d_p}{d_d}$.

## D. Determination of Molecular Weight by Gel Filtration on Sephadex G-100.

Sephadex G-100 (lot No. TO 5967, particle size 40-120 mesh) and Calibration Kit proteins (Kit No. 9KA), were obtained from Pharmacia, Uppsala, Sweden. Sephadex G-100 gel filtration media, used in all experiments for molecular weight determinations, was added to excess phosphate buffer, pH 6.9, containing 0.05 M NaCl, and the gel was allowed to swell for at least 3 days. The swollen gel was stirred and settled several times from the buffer in order to remove the very fine particles. The resultant gel was packed into a 2.5 x 45 cm. column (Pharmacia K25/45, bed volume 292 ml.) with two flow adaptors. The column was then washed with buffer for two days to be completely equilibrated as judged by the constant elution volume of Blue Dextran gel.

The dried sample was added to 1.0 ml. phosphate buffer, pH 6.9, containing 0.05 M NaCl, and centrifuged at 7000 rpm. for 10 min. to be free from insoluble materials. The resulting supernatant was applied through the 3-way valve which attached to the lower flow adaptor and the protein was washed into the gel

with the buffer solution at an upward flow rate of 22.5 ml./hr. Fractions of 1.5 ml. were collected with an Isco fraction collector equipped with a timing device. Protein contents of the fractions were determined spectrophotometrically at 280 mμ. All the experiments were done at 5° C. Elution volumes were interpolated to the nearest 0.1 ml. by triangulation.

The void volume, $V_o$, was determined by applying to the column 0.5 ml. of 0.5 percent Blue Dextran, freshly prepared in phosphate buffer, and measuring the effluent volume from the initial application of Blue Dextran sample to the appearance of the blue color in the effluent.

The selectivity curve was prepared by using the Calibration Kit Standards. The molecular weights and xources of the Calibration Kit proteins are:

| Protein Standard | Molecular Weight | Source |
|---|---|---|
| aldolase (lot No. 9GA) | 158,000 | rabbit muscle |
| ovalbumin (lot No. 8CA) | 45,000 | egg white |
| chymotrypsinogen A (lot No. 8HA) | 25,000 | bovine pancreas |
| ribonuclease A (lot No. 9CA) | 13,700 | bovine pancreas |

The mixtures of 30 mg. aldolase and 10 mg. chymotrypsinogen A; and 20 mg. each of ovalbumin and ribonuclease A were taken up in separate 1 ml. buffer volumes and chromatographed separately, using the same conditions as for all other experiments on the column. Since the total bed volume and void volume were known, the calculation of $K_{av}$ for each protein standard was performed by using the equation,

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

where $V_e$ = elution volume for the protein

$V_o$ = elution volume for Blue Dextran 2000

$V_t$ = total bed volume.

The plot of the $K_{av}$ value for each protein standard against the corresponding logarithm of the molecular weight was used as the standard curve from which the unknown molecular weight of a protein can be estimated directly when the $K_{av}$ value of that protein has been determined.

## E. Determination of Amino Acids

The chromatographic analysis of amino acids was carried out in the Department of Biochemistry on a Beckman Spinco model 120B amino acid analyzer using columns packed with Beckman Type AA-15 and PA35 resins and operated at the flow rates of 35 ml. ninhydrin reagent and 70 ml. buffer per hour. Ninhydrin reagent for the color development with amino acids was prepared by dissolving 80.0 g. ninhydrin in 4 liters of solvent consisting of 75 percent methyl Cello-solve and 25 percent 4 M sodium acetate buffer (pH 5.51). The samples for chromatographic analysis of amino acids were prepared in the following manner:

One milligram of lyophilyzed rapeseed protein fraction was hydrolyzed with 1 ml of 6 N constant boiling hydrochloric acid for 18 hr. at 110°C in an evacuated, sealed tube. The hydrolyzate was evaporated to dryness in a vacuum desiccator in the presence of sodium hydroxide. The dried hydrolyzate was dissolved in 5 ml.

of sodium citrate buffer of pH 2.2 and 2 ml. of the solution was used for the determination of the acidic and neutral amino acids, and another 2 ml. for the basic amino acid analyses.

# IV. RESULTS

## A. Standard Kit Curves for Molecular Weight Determination on Sephadex

To avoid overlapping of the standard protein mixtures, the determination of their elution volumes was conducted in two phases. The elution patterns of the pure proteins are shown in Figure 1. Elution volumes obtained for aldolase, albumin, chymotrypsinogen A and ribonuclease A were 65.6, 99.1, 126.1 and 146.2 ml. respectively. Blue dextran 2000 gave a very sharp curve with an elution volume ($V_o$) of 51.1 ml. The calculation of the $K_{av}$ (elution constant) values for the proteins was carried out according to the formula,

$K_{av} = \frac{V_e - V_o}{V_t - V_o}$, and are as follows:

$K_{av}$ of aldolase, $\frac{65.6 - 51.1}{291.9 - 51.1} = 0.0602$ (M.W. = 158,000)

$K_{av}$ of ovalbumin, $\frac{99.1 - 51.1}{291.9 - 51.1} = 0.1997$ (M.W. = 45,000)

$K_{av}$ of chymotrypsinogen A, $\frac{126.0 - 51.1}{291.9 - 51.1} = 0.3110$

(M.W. = 25,000)

$K_{av}$ of ribonuclease A, $\frac{146.2 - 51.1}{291.9 - 51.1} = 0.3990$

(M.W. = 13,700)

A plot of $K_{av}$ values versus the logarithm of molecular weights of the Calibration Kit proteins is presented in Figure 2. The relationship was found to be linear in the molecular weight

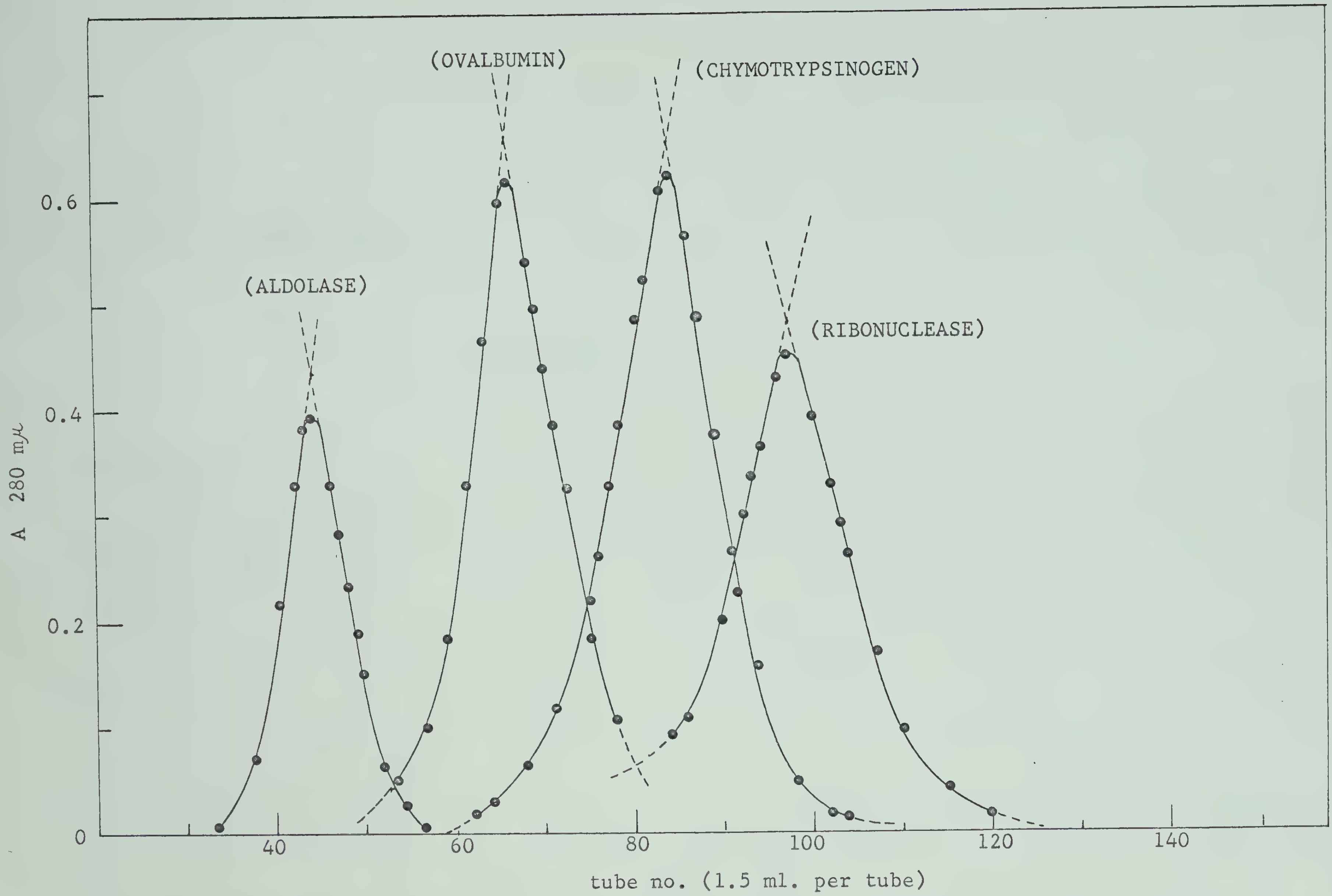


Figure 1. Elution curves for Calibration Kit proteins on Sephadex G-100. Eluant used, phosphate buffer pH 6.9, containing 0.05 M sodium chloride.

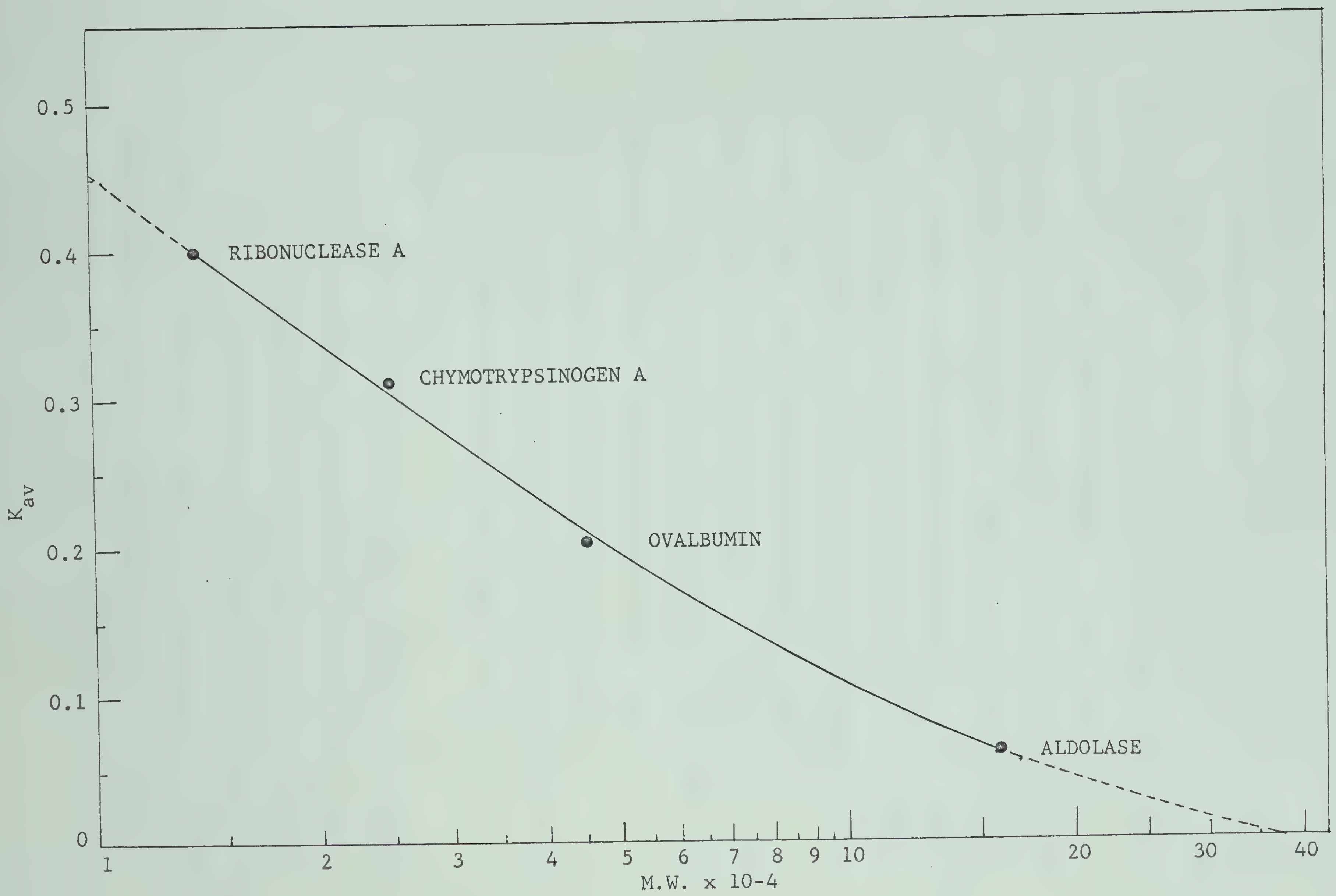


Figure 2. Selectivity curve for Sephadex G-100 column. Molecular weights; ribonuclease A - 13,700, chymotrypsinogen A - 25,000, ovalbumin - 45,000, and aldolase - 158,000.

range from 13,000 - 45,000.

## B. Solvent Systems and Yield of Each

Different solvents were used to extract various classes of rapeseed proteins. Ten percent sodium chloride, 0.01 M sodium pyrophosphate, 0.05 M acetic acid and 0.2 percent (W/V) sodium hydroxide were the solvents of choice for the purpose of investigating the effectiveness of extraction, because they have been widely used for studies on other seed proteins. As well, sodium chloride and sodium pyrophosphate are in use in other areas of food processing. Acetic acid was employed, as the globular protein bodies from most aleurone grains have been shown to be soluble in this acid. Sodium hydroxide may be capable of extracting those proteins which are not extractable by the other solvents. The yields of protein extracted by each solvent are summarized in Table 2. The results show that each of these extracting solutions had a different solubilizing effect on the rapeseed proteins. The percentages of the protein obtained from Lowry protein determination are lower than the yields calculated on dry weight basis.

Dialysis of the extracts against appropriate dialyzing solutions, to eliminate the dialyzable compounds other than proteins, caused the precipitation of some protein components. The soluble and insoluble proteins obtained after lyophilization varied in color from rather white in the supernantant fraction of the acetic acid extract to a dark brown for the precipitated fraction from the sodium chloride. The schematic diagrams of the

Table 2

Yield of protein from different extracting solutions

| Extracting solutions | Protein Content (%) | | | | | |
|---|---|---|---|---|---|---|
| | Dry weight basis | | | Lowry-protein determination | | |
| | Soluble after dialysis | Precipitated on dialysis | Total | Soluble after dialysis | Precipitated on dialysis | Total |
| 0.01 M sodium pyrophosphate (pH 7.0) | 17.6 | 10.2 | 27.8 | 9.8 | 4.4 | 14.2 |
| 10 percent sodium chloride (W/V) | 9.1 | 12.9 | 21.0 | 5.6 | 9.5 | 15.1 |
| 0.05 M acetic acid (pH 3.1) | 3.2 | 1.8 | 5.0 | 1.0 | 0.9 | 1.9 |
| 0.2 percent sodium hydroxide (W/V) | 39.0 | - | 39.0 | 20.5 | - | 20.5 |

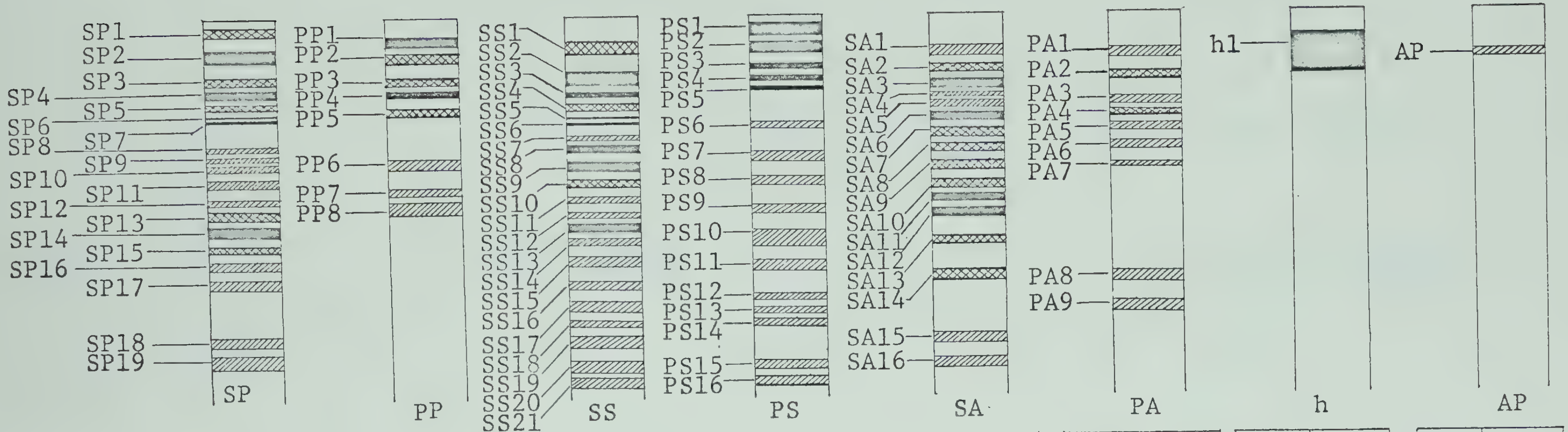
electrophoretic patterns of the soluble and insoluble fractions for all solvents are shown in Figure 3, together with the $R_p$ value tables, revealing many bands stained to various intensities in the neutral salt and acetic acid extracts, but only one thick band detectable in the protein extracted with sodium hydroxide. These differences may be indicative of the concentration of the respective protein constituents or may be a manifestation of absorbability of the stain by the proteins. Only one faint band was shown when acetic acid was used subsequent to extraction with sodium pyrophosphate. Of all the insoluble fractions, it is generally considered that many of the bands are identical to those observed in the soluble fractions. The distinct bands observed to have no corresponding $R_p$ values in the insoluble fractions from sodium pyrophosphate, sodium chloride, and acetic acid are defined as PP1; PS1, PS3, PS8, PS13; and PA2, PA3, PA6 and PA9 respectively.

## C. Characteristics of Proteins from Each Solvent System

### (1) Protein extracted with 0.01 M sodium pyrophosphate

#### a. Sephadex chromatography and molecular weight determination.

The results of the chromatography of protein extracted with 0.01 M sodium pyrophosphate are shown in Figure 4. The SP1 fraction appears to be the major constituent of the soluble fraction. Five fractions, SPI, SPII, SPIII, SPIV and SPV were obtained from the soluble material and two fractions, PPI and PPII were obtained



| Bands | $R_p$ |
|---|---|
| SP1 | .0240 |
| SP2 | .0700 |
| SP3 | .1266 |
| SP4 | .1471 |
| SP5 | .1676 |
| SP6 | .1891 |
| SP7 | .1991 |
| SP8 | .2487 |
| SP9 | .2612 |
| SP10 | .2792 |
| SP11 | .3618 |
| SP12 | .3333 |
| SP13 | .3663 |
| SP14 | .4054 |
| SP15 | .4234 |
| SP16 | .4594 |
| SP17 | .4984 |
| SP18 | .6186 |
| SP19 | .6516 |

| Bands | $R_p$ |
|---|---|
| PP1 | .0458 |
| PP2 | .0728 |
| PP3 | .1213 |
| PP4 | .1455 |
| PP5 | .1807 |
| PP6 | .2762 |
| PP7 | .3234 |
| PP8 | .3530 |

| Bands | $R_p$ |
|---|---|
| SS1 | .0645 |
| SS2 | .1218 |
| SS3 | .1489 |
| SS4 | .1752 |
| SS5 | .1998 |
| SS6 | .2091 |
| SS7 | .2329 |
| SS8 | .2564 |
| SS9 | .2734 |
| SS10 | .3138 |
| SS11 | .3416 |
| SS12 | .3728 |
| SS13 | .4012 |
| SS14 | .4225 |
| SS15 | .4672 |
| SS16 | .5128 |
| SS17 | .5468 |
| SS18 | .5996 |
| SS19 | .6108 |
| SS20 | .6667 |
| SS21 | .6850 |

| Bands | $R_p$ |
|---|---|
| PS1 | .0360 |
| PS2 | .0610 |
| PS3 | .0880 |
| PS4 | .1198 |
| PS5 | .1422 |
| PS6 | .2255 |
| PS7 | .2750 |
| PS8 | .3266 |
| PS9 | .3651 |
| PS10 | .4245 |
| PS11 | .4768 |
| PS12 | .5468 |
| PS13 | .5630 |
| PS14 | .6080 |
| PS15 | .6669 |
| PS16 | .6850 |

| Bands | $R_p$ |
|---|---|
| SA1 | .1059 |
| SA2 | .1247 |
| SA3 | .1512 |
| SA4 | .1662 |
| SA5 | .1973 |
| SA6 | .2188 |
| SA7 | .2454 |
| SA8 | .2568 |
| SA9 | .2838 |
| SA10 | .3241 |
| SA11 | .3483 |
| SA12 | .3947 |
| SA13 | .4057 |
| SA14 | .5069 |
| SA15 | .6354 |
| SA16 | .6803 |

| Bands | $R_p$ |
|---|---|
| PA1 | .1097 |
| PA2 | .1455 |
| PA3 | .1810 |
| PA4 | .1973 |
| PA5 | .2225 |
| PA6 | .2551 |
| PA7 | .2863 |
| PA8 | .5014 |
| PA9 | .5489 |

| Band | $R_p$ |
|---|---|
| h1 | .0752 |

| Band | $R_p$ |
|---|---|
| AP | .0795 |

Figure 3. Schematic diagrams of electrophoretograms of rapeseed proteins. (SP soluble in pyrophosphate, PP precipitated on dialysis of pyrophosphate, SS soluble in sodium chloride, PS precipitated on dialysis of sodium chloride, SA soluble in acetic acid, PA precipitated on dialysis of acetic acid, h soluble in sodium hydroxide, AP acetic acid soluble after pyrophosphate extraction)

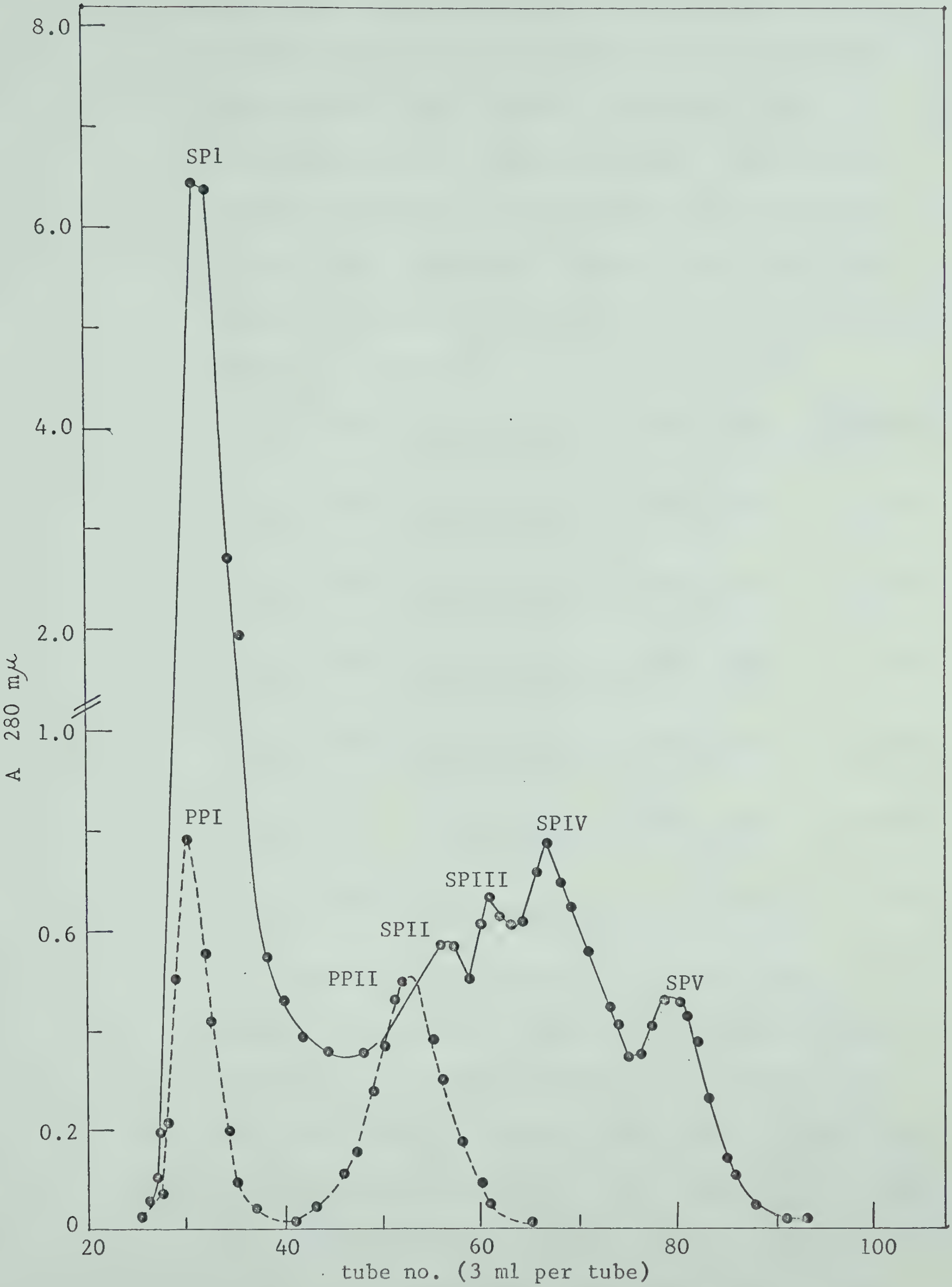


Figure 4. Elution curves on Sephadex G-100 of proteins extracted with 0.01 M sodium pyrophosphate, pH 7.0. Eluant used, borate buffer pH 8.6.

——— Soluble fractions

----- Insoluble fractions

from the material precipitated during dialysis. In Figure 5, the elution curves for the molecular weight determination of each fraction separated by gel filtration on Sephadex G-100 are shown. The elution volumes of 63.4, 110.7, 134.2 196.6, 215.5, 63.0 and 136.2 ml. were obtained for fractions SPI, SPII, SPIII, SPIV, SPV, PPI and PPII respectively. The $K_{av}$ values are determined as follows:

$$K_{av} \text{ of SPI, } \quad \frac{63.5 - 51.1}{291.9 - 51.1} = 0.0514 \text{ (M.W. = 170,000)}$$

$$K_{av} \text{ of SPII, } \quad \frac{110 - 51.1}{291.9 - 51.1} = 0.2475 \text{ (M.W. = 35,000)}$$

$$K_{av} \text{ of SPIII, } \quad \frac{134.2 - 51.1}{291.9 - 51.1} = 0.3453 \text{ (M.W. = 19,000)}$$

$$K_{av} \text{ of SPIV, } \quad \frac{196.6 - 51.1}{291.9 - 51.1} = 0.6044 \text{ (M.W. } < 10{,}000\text{)}$$

$$K_{av} \text{ of SPV, } \quad \frac{215.8 - 51.1}{291.9 - 51.1} = 0.6842 \text{ (M.W. } < 10{,}000\text{)}$$

$$K_{av} \text{ of PPI, } \quad \frac{63.0 - 51.1}{291.9 - 51.1} = 0.0494 \text{ (M.W. = 175,000)}$$

$$K_{av} \text{ of PPII, } \quad \frac{136.2 - 51.1}{291.9 - 51.1} = 0.3534 \text{ (M.W. = 18,500)}$$

The molecular weights of SPI, SPII, SPIII, PPI and PPII calculated from their $K_{av}$ values were 170,000, 35,000, 19,000, 175,000 and 18,500 respectively. The molecular weight of fractions SPIV and SPV are less than 10,000 and outside the range of the standard curve. The results of molecular weight determinations indicates the similarity of fraction SPI to PPI, and SPIII to PPII.

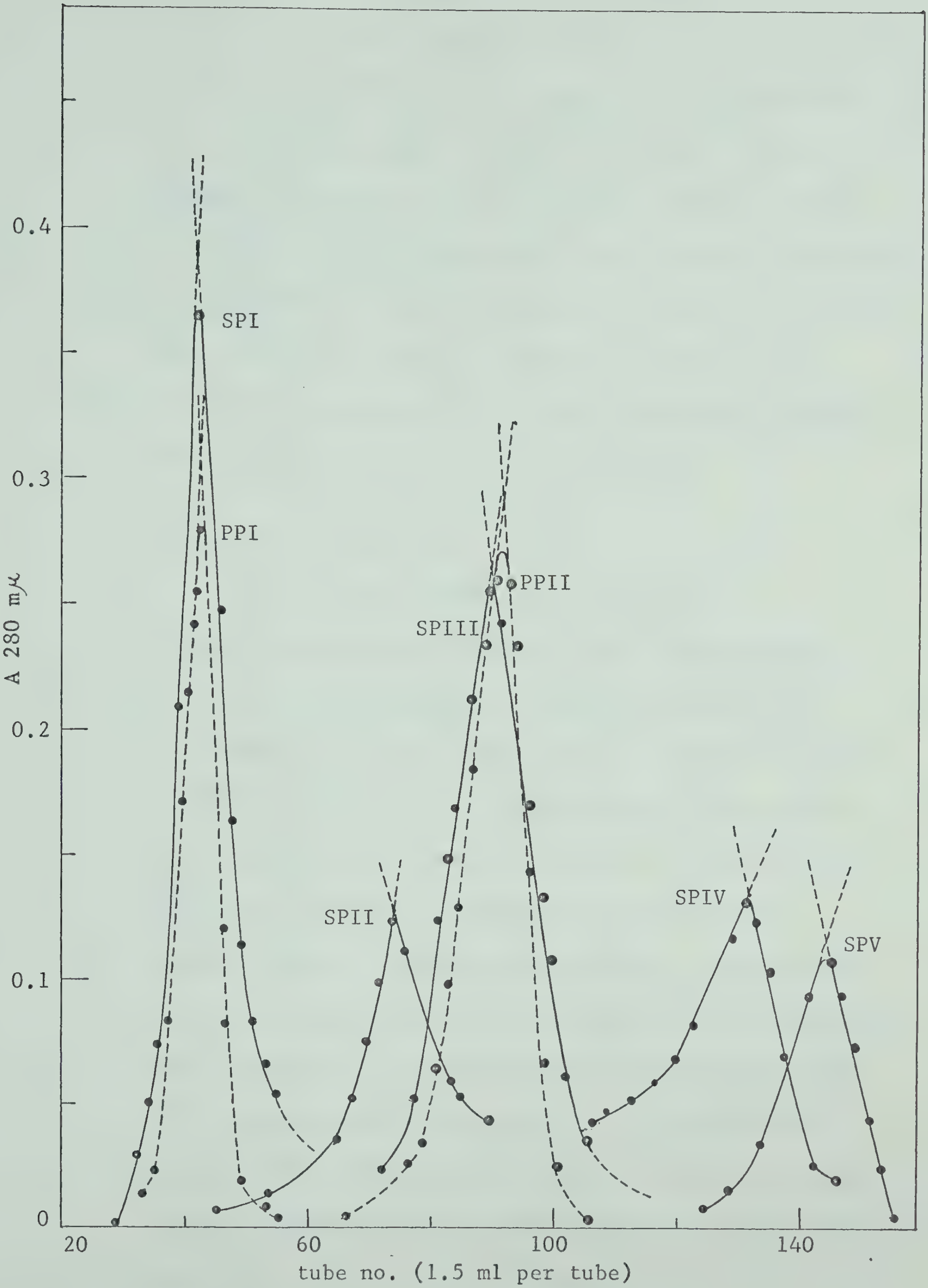


Figure 5. Elution curves on Sephadex G-100 for molecular weight determinations of proteins extracted with 0.01 M sodium pyrophosphate, pH 7.0. Eluant used, phosphate buffer, pH 6.9, containing 0.05 M sodium chloride.

——— Soluble fractions

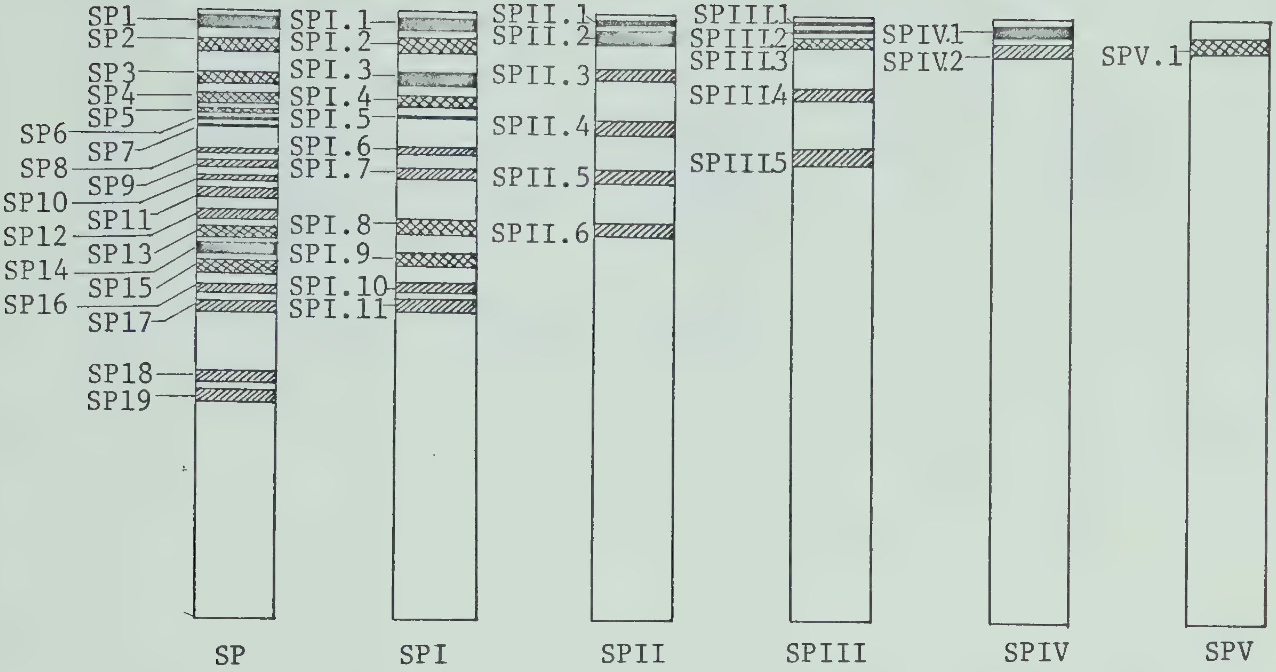
----- Insoluble fractions

b. Polyacrylamide disc gel electrophoresis.

Electrophoretograms of SPI, SPII, SPIII, SPIV and SPV (Fig. 6) show 11, 6, 5, 2 and 1 bands respectively. The protein constituent having the $R_p$ value 0.0558 appears homogeneous in fraction SPV. Results of polyacrylamide gel electrophoresis of the two fractions from the insoluble material are given in Figure 7. Six bands were detected in fraction PPI whereas 2 bands were observed in fraction PPII. Most of the bands present in PPI are identical to those present in SPI.

c. Amino acid analyses

Table 3 lists amino acid analyses of fractionated proteins from the soluble and insoluble portions of the pyrophosphate buffer, expressed as percentage of the total amino acids. Comparisons of the values show the variation of some amino acids, generally in the aspartic acid, proline, glycine, valine, tyrosine and lysine contents. Fraction SPV is deficient in cystine, lysine, histidine and arginine. Obviously low proline and high methionine were observed in the fraction PPI. Distribution of amino acids in fractions SPII and SPIII are essentially the same as the distribution in fraction PPII. The only difference is that fraction PPII contains lower amounts of lysine and histidine than SPII and SPIII.



| Bands | $R_p$ |
|---|---|
| SP1 | .024 |
| SP2 | .070 |
| SP3 | .1266 |
| SP4 | .1471 |
| SP5 | .1676 |
| SP6 | .1891 |
| SP7 | .1991 |
| SP8 | .2487 |
| SP9 | .2612 |
| SP10 | .2792 |
| SP11 | .3068 |
| SP12 | .3333 |
| SP13 | .3663 |
| SP14 | .4054 |
| SP15 | .4234 |
| SP16 | .4594 |
| SP17 | .4984 |
| SP18 | .6186 |
| SP19 | .6516 |

| Bands | $R_p$ |
|---|---|
| SPI.1 | .0258 |
| SPI.2 | .0738 |
| SPI.3 | .1255 |
| SPI.4 | .1587 |
| SPI.5 | .1846 |
| SPI.6 | .2423 |
| SPI.7 | .2769 |
| SPI.8 | .3618 |
| SPI.9 | .4246 |
| SPI.10 | .4504 |
| SPI.11 | .4726 |

| Bands | $R_p$ |
|---|---|
| SPII.1 | .0250 |
| SPII.2 | .0531 |
| SPII.3 | .1218 |
| SPII.4 | .1953 |
| SPII.5 | .2828 |
| SPII.6 | .3812 |

| Bands | $R_p$ |
|---|---|
| SPIII.1 | .020 |
| SPIII.2 | .0414 |
| SPIII.3 | .0582 |
| SPIII.4 | .1442 |
| SPIII.5 | .2643 |

| Bands | $R_p$ |
|---|---|
| SPIV.1 | .0375 |
| SPIV.2 | .0609 |

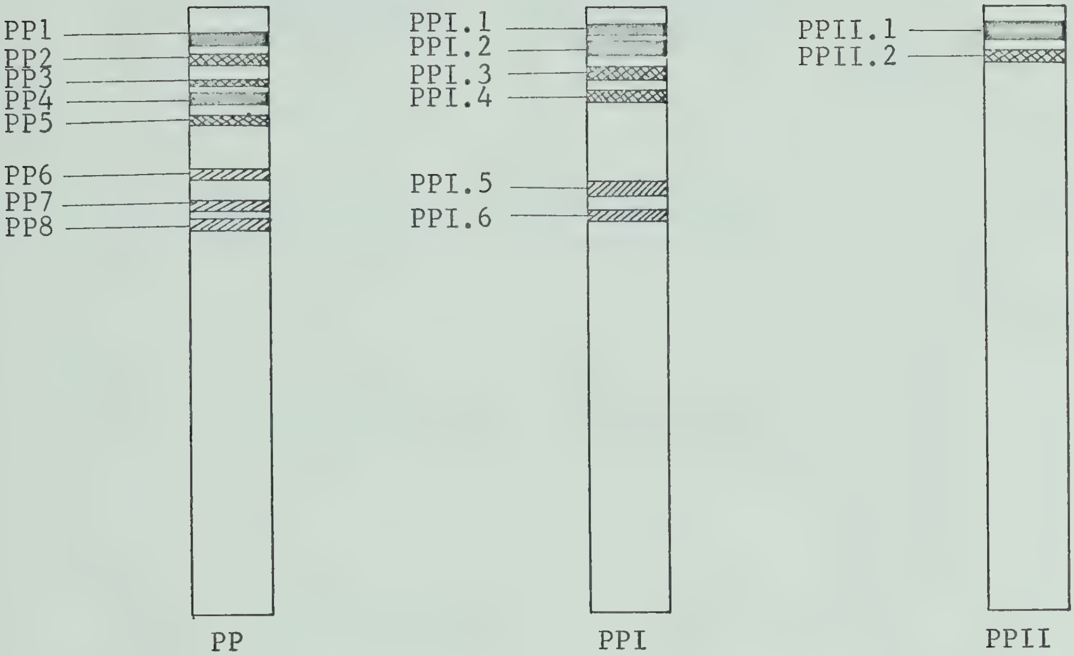
| Bands | $R_p$ |
|---|---|
| SPV.1 | .0558 |

▨ —— faint

▩ —— medium

■ —— intense

Figure 6. Schematic diagrams of electrophoretograms of separated soluble fractions of proteins extracted with 0.01 M sodium pyrophosphate.



| Bands | $R_p$ |
|---|---|
| PP1 | .0458 |
| PP2 | .0728 |
| PP3 | .1213 |
| PP4 | .1455 |
| PP5 | .1807 |
| PP6 | .2736 |
| PP7 | .2930 |
| PP8 | .3531 |

| Bands | $R_p$ |
|---|---|
| PPI.1 | .0227 |
| PPI.2 | .0530 |
| PPI.3 | .1212 |
| PPI.4 | .1439 |
| PPI.5 | .2954 |
| PPI.6 | .3590 |

| Bands | $R_p$ |
|---|---|
| PPII.1 | .0415 |
| PPII.2 | .0763 |

Figure 7. Schematic diagrams of electrophoretograms of separated insoluble fractions of proteins extracted with 0.01 M sodium pyrophosphate.

Table 3

Amino acid analyses of rapeseed protein extracted with pyrophosphate buffer (expressed as percent of total amino acid)

| Amino acids | Gel filtration fractions | | | | | | |
|---|---|---|---|---|---|---|---|
| | SPI | SPII | SPIII | SPIV | SPV | PPI | PPII |
| Aspartic acid | 10.70 | 3.13 | 2.60 | 2.83 | 5.99 | 9.95 | 2.23 |
| Threonine | 4.70 | 3.61 | 3.22 | 3.34 | 4.17 | 4.39 | 3.56 |
| Serine | 4.50 | 4.50 | 4.05 | 4.33 | 7.18 | 4.82 | 4.61 |
| Glutamic acid | 20.00 | 25.87 | 27.30 | 27.40 | 29.76 | 21.08 | 31.65 |
| Proline | 5.11 | 10.20 | 9.46 | 9.65 | 12.92 | 0.65 | 11.28 |
| Glycine | 5.86 | 4.33 | 3.98 | 4.40 | 8.34 | 4.24 | 2.78 |
| Alanine | 5.05 | 4.02 | 3.97 | 3.13 | 5.67 | 4.76 | 4.41 |
| Cystine | 0.90 | 4.11 | 5.61 | 5.59 | - | - | 5.58 |
| Valine | 5.94 | 7.04 | 4.97 | 5.49 | 4.44 | 6.03 | 4.92 |
| Methionine | 2.54 | 2.07 | 2.43 | 1.48 | 2.17 | 8.22 | 2.83 |
| Isoleucine | 4.67 | 3.13 | 3.36 | 3.35 | 4.52 | 4.70 | 3.63 |
| Leucine | 8.40 | 7.08 | 6.84 | 6.66 | 8.58 | 8.10 | 7.71 |
| Tyrosine | 3.42 | 1.52 | 1.28 | trace | 1.82 | 3.27 | 1.19 |
| Phenylalanine | 4.59 | 3.15 | 3.35 | 3.30 | 4.39 | 4.86 | 3.31 |
| Lysine | 3.70 | 6.75 | 7.85 | 8.29 | trace | 5.03 | 4.54 |
| Histidine | 2.66 | 3.71 | 3.83 | 4.13 | trace | 2.61 | 2.15 |
| Arginine | 7.08 | 5.70 | 5.81 | 6.53 | trace | 7.21 | 3.55 |

SP Soluble fraction on dialysis of protein extracted with 0.01 M sodium pyrophosphate

PP Insoluble fraction on dialysis of protein extracted with 0.01 M sodium pyrophosphate

### (2) Protein extracted in 10 percent Sodium Chloride.

#### a. Sephadex chromatography and molecular weight determination.

The chromatography of the dialyzed sodium chloride extract, soluble (SS) and insoluble (PS) portions, resulted in three and two fractions respectively. The chromatographs are presented in Figure 8. The characteristics of the chromatogram indicates that SSII was composed of a large amount of protein which is water soluble. The distribution of the soluble and insoluble protein eluted with phosphate buffer, (pH 6.5), containing 0.05 M sodium chloride are presented in Figure 9. Fractions SSI, SSII, SSIII, PSI and PSII have elution volumes of 72.0, 135.0, 205.0, 65.5 and 113.7 ml, respectively. The calculation for the $K_{av}$ values of each isolated fraction is shown below:

$K_{av}$ of SSI, $\frac{72.0 - 51.1}{291.9 - 51.1} = 0.0867$ (M.W.=115,000)

$K_{av}$ of SSII, $\frac{135.0 - 51.1}{291.9 - 51.1} = 0.3484$ (M.W.= 19,000)

$K_{av}$ of SSIII, $\frac{205.5 - 51.1}{291.9 - 51.1} = 0.6393$ (M.W. < 10,000)

$K_{av}$ of PSI, $\frac{64.5 - 51.1}{291.9 - 51.1} = 0.0556$ (M.W.=160,000)

$K_{av}$ of PSII, $\frac{113.7 - 51.1}{291.9 - 51.1} = 0.2598$ (M.W.= 32,500)

According to the $K_{av}$ values, the molecular weights of these fractions were found to be 115,000, 19,000, 160,000, 32,500 for SSI, SSII, PSI and PSII

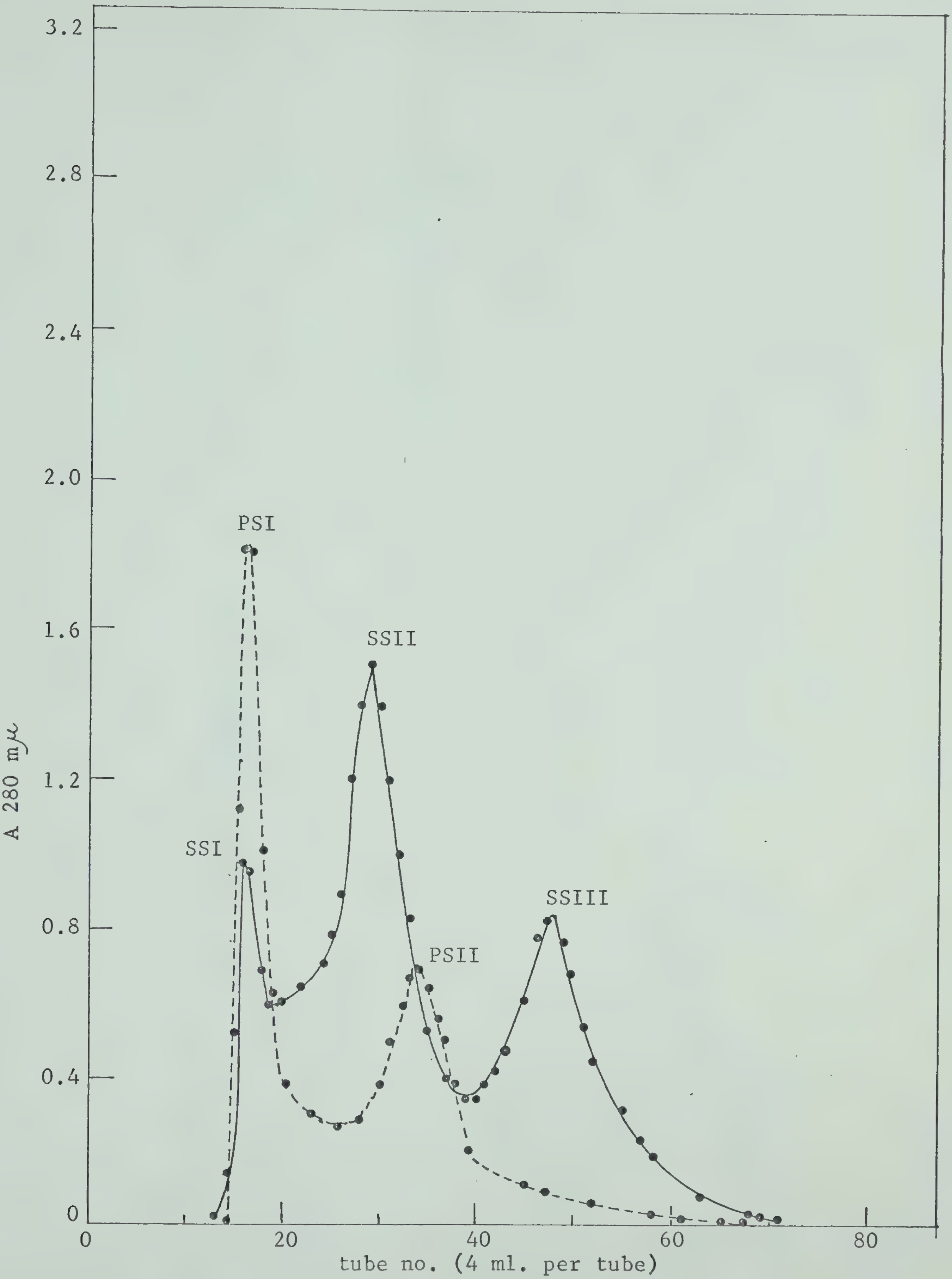


Figure 8. Elution curves on Sephadex G-100 of protein extracted with 10 percent sodium chloride. Eluant used, borate buffer pH 8.6, containing 5 percent sodium chloride.

——— Soluble fractions

----- Insoluble fractions

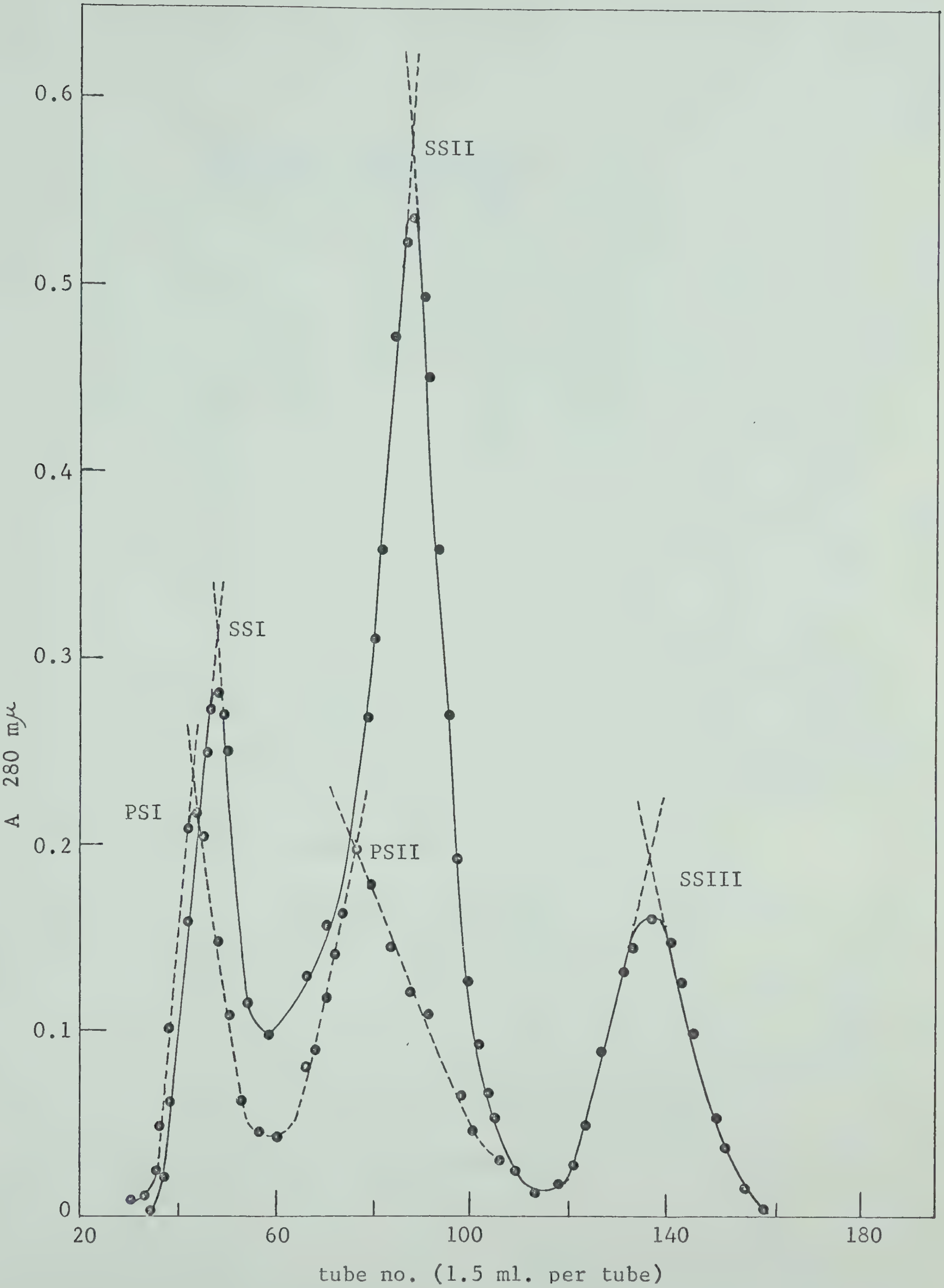


Figure 9. Elution curves on Sephadex G-100 for molecular weight determinations of protein extracted with 10 percent sodium chloride. Eluant used, phosphate buffer pH 6.9, containing 0.05 M sodium chloride.

——— Soluble fractions

----- Insoluble fractions

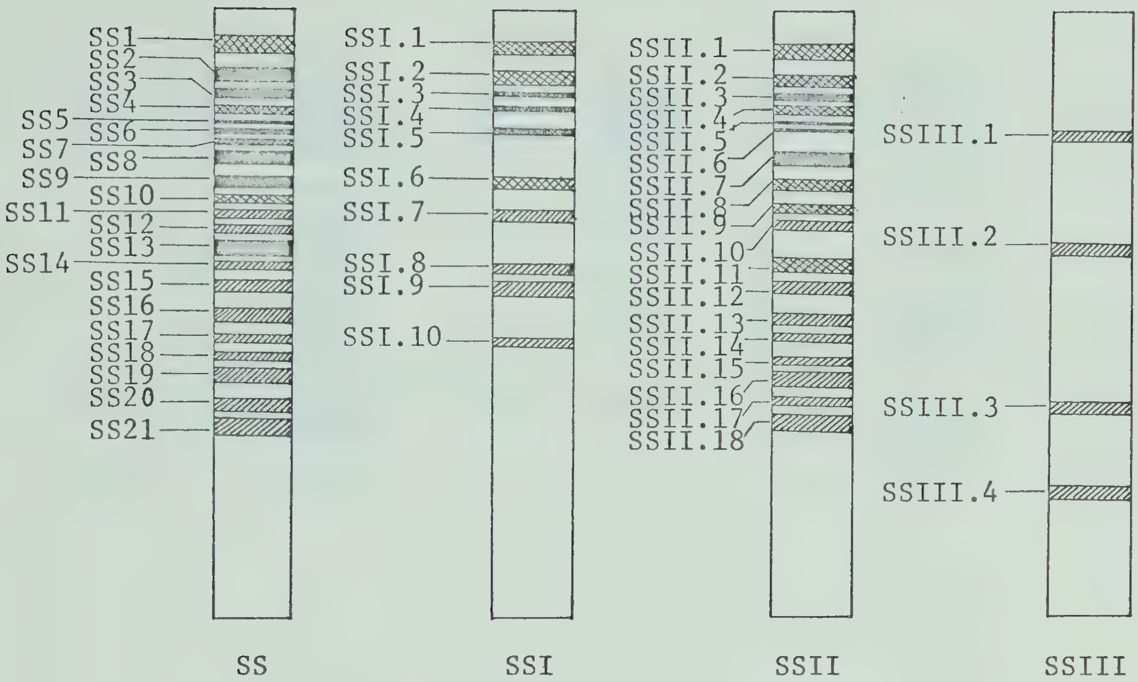
respectively, and the molecular weight of SSIII is lower than 10,000.

### b. Polyacrylamide disc gel electrophoresis of 10 percent sodium chloride extract.

The electrophoretic patterns of fractionated proteins extracted with 10 percent sodium chloride show 10, 18 and 4 components for the gels SSI, SSII and SSIII (Fig. 10) and 9 bands for both PSI and PSII gels (Fig. 11). The constituents of SSII are almost the same as those detected in the fractionated soluble fraction. The bands SSI.1, SSI.2, SSI.3, SSI.4, SSI.5, SSI.6, SSI.9 and SSI.10 are identical to SSII.1, SSII.2, SSII.3, SSII.4, SSII.6, SSII.8, SSII.12 and SSII.14 - respectively and SSII.16 is similar to SSIII.3. Proteins PSI.4 and PSI.7 are correspondingly similar to PSII.3 and PSII.5.

### c. Amino acid analysis

The amino acid analysis of all fractions of the NaCl extraction are presented in Table 4. The insoluble proteins, PSI and PSII, contain lower amounts of basic amino acids than the soluble proteins. In the case of all the three soluble fractions the amino acid concentrations are comparable.



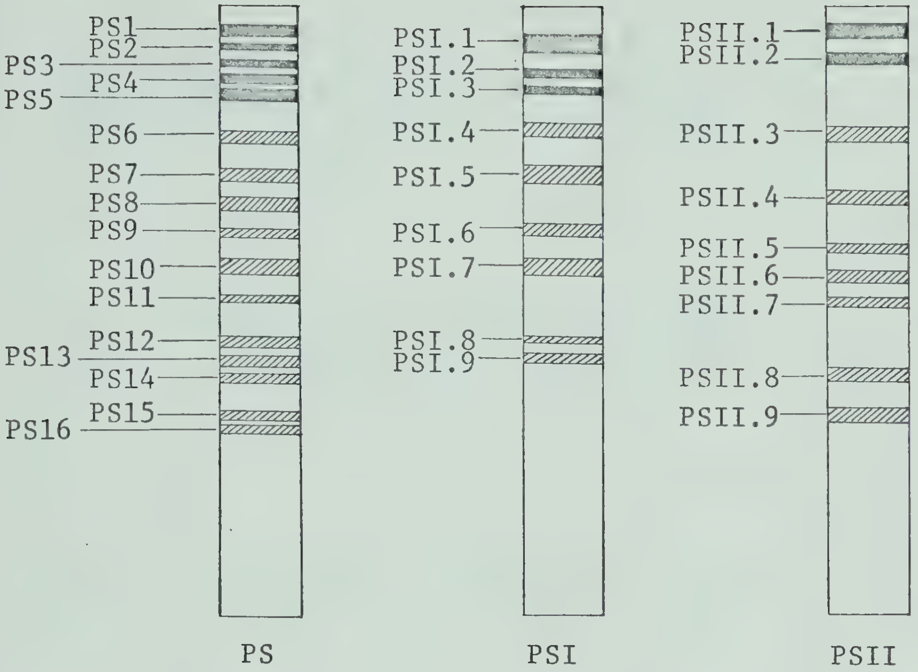
| Bands | $R_p$ |
|---|---|
| SS1 | .0645 |
| SS2 | .1218 |
| SS3 | .1489 |
| SS4 | .1752 |
| SS5 | .1998 |
| SS6 | .2091 |
| SS7 | .2329 |
| SS8 | .2564 |
| SS9 | .2734 |
| SS10 | .3238 |
| SS11 | .3416 |
| SS12 | .3228 |
| SS13 | .4012 |
| SS14 | .4225 |
| SS15 | .4672 |
| SS16 | .5128 |
| SS17 | .5497 |
| SS18 | .5824 |
| SS19 | .6108 |
| SS20 | .6576 |
| SS21 | .6866 |

| Bands | $R_p$ |
|---|---|
| SSI.1 | .0650 |
| SSI.2 | .1232 |
| SSI.3 | .1458 |
| SSI.4 | .1752 |
| SSI.5 | .2063 |
| SSI.6 | .2787 |
| SSI.7 | .3458 |
| SSI.8 | .4218 |
| SSI.9 | .4538 |
| SSI.10 | .5445 |

| Bands | $R_p$ |
|---|---|
| SSII.1 | .0647 |
| SSII.2 | .1230 |
| SSII.3 | .1514 |
| SSII.4 | .1735 |
| SSII.5 | .1908 |
| SSII.6 | .2050 |
| SSII.7 | .2539 |
| SSII.8 | .2918 |
| SSII.9 | .3249 |
| SSII.10 | .3738 |
| SSII.11 | .4247 |
| SSII.12 | .4653 |
| SSII.13 | .5142 |
| SSII.14 | .5457 |
| SSII.15 | .6088 |
| SSII.16 | .6566 |
| SSII.17 | .6878 |

| Bands | $R_p$ |
|---|---|
| SSIII.1 | .2288 |
| SSIII.2 | .4012 |
| SSIII.3 | .6489 |
| SSIII.4 | .7899 |

Figure 10. Schematic diagrams of electrophoretograms of separated soluble fractions of proteins extracted with 10 percent sodium chloride.



| Bands | $R_p$ |
|---|---|
| PS1 | .0360 |
| PS2 | .0610 |
| PS3 | .0880 |
| PS4 | .1198 |
| PS5 | .1422 |
| PS6 | .2255 |
| PS7 | .2750 |
| PS8 | .3266 |
| PS9 | .3651 |
| PS10 | .4245 |
| PS11 | .4768 |
| PS12 | .5468 |
| PS13 | .5642 |
| PS14 | .5996 |
| PS15 | .5667 |
| PS16 | .6850 |

| Bands | $R_p$ |
|---|---|
| PSI.1 | .0622 |
| PSI.2 | .1240 |
| PSI.3 | .1470 |
| PSI.4 | .2131 |
| PSI.5 | .2778 |
| PSI.6 | .3636 |
| PSI.7 | .4130 |
| PSI.8 | .5403 |
| PSI.9 | .5683 |

| Bands | $R_p$ |
|---|---|
| PSII.1 | .0338 |
| PSII.2 | .0775 |
| PSII.3 | .2229 |
| PSII.4 | .3236 |
| PSII.5 | .4042 |
| PSII.7 | .4839 |
| PSII.8 | .5908 |
| PSII.9 | .6315 |

Figure 11. Schematic diagrams of electrophoretograms of separated insoluble fractions of proteins extracted with 10 percent sodium chloride.

Table 4.

Amino acid analyses of rapeseed protein extracted with sodium chloride (expressed as percent of total amino acid)

| Amino acids | Gel filtration fractions | | | | |
|---|---|---|---|---|---|
| | SSI | SSII | SSIII | PSI | PSII |
| Aspartic acid | 10.36 | 5.55 | 12.52 | 11.64 | 3.41 |
| Threonine | 5.76 | 4.29 | 4.86 | 4.71 | 3.51 |
| Serine | 6.09 | 4.51 | 4.81 | 5.07 | 4.52 |
| Glutamic acid | 12.66 | 22.79 | 21.10 | 23.85 | 29.60 |
| Proline | 4.95 | 8.14 | 6.00 | 6.39 | 10.77 |
| Glycine | 5.25 | 5.00 | 6.43 | 6.37 | 4.96 |
| Alanine | 4.60 | 4.47 | 4.33 | 4.89 | 4.27 |
| Cystine | 0.37 | 3.46 | trace | 0.88 | 5.21 |
| Valine | 5.90 | 4.87 | 3.35 | 4.72 | 5.60 |
| Methionine | 2.44 | 2.29 | 1.76 | 2.31 | 2.79 |
| Isoleucine | 4.91 | 3.51 | 3.78 | 4.74 | 4.01 |
| Leucine | 8.33 | 6.79 | 5.47 | 9.39 | 7.63 |
| Tyrosine | 4.15 | 3.02 | 3.31 | 2.60 | 1.94 |
| Phenylalanine | 5.88 | 3.43 | 3.80 | 5.45 | 3.91 |
| Lysine | 9.53 | 8.59 | 8.14 | 2.47 | 3.42 |
| Histidine | 2.53 | 3.55 | 2.74 | 1.11 | 1.63 |
| Arginine | 5.99 | 5.79 | 5.57 | 3.32 | 2.72 |

SS Soluble fraction on dialysis of protein extracted with 10 percent NaCl.

PS Insoluble fraction on dialysis of protein extracted with 10 percent NaCl.

### (3) Protein extracted with 0.05 M acetic acid.

#### a. Sephadex gel filtration and molecular weight determination

The protein extracted from the meal with 0.05 M acetic acid (pH 3.1), both supernatant and fraction precipitated upon dialysis against 0.005 M acetate buffer (pH 4.1), were chromatographed on Sephadex G-100 using 0.005 M acetate buffer as the eluant. A plot of optical density vs. fraction number (Fig. 12) showed similar elution patterns containing two peaks in both the soluble (solid line) and insoluble (dotted line) fractions. In the determination of the molecular weights of these fractions (Fig. 13), the elution volumes of 59.1, 137.1, 62.0 and 134.7 ml. were found for SAI, SAII, (the soluble fraction), PAI and PAII (the insoluble fraction), respectively. The $K_{av}$ values are determined below as:

$$K_{av} \text{ of SAI,} \quad \frac{59.1 - 51.1}{291.9 - 51.1} = 0.0332 \text{ (M.W. = 220,000)}$$

$$K_{av} \text{ of SAII,} \quad \frac{137.1 - 51.1}{291.9 - 51.1} = 0.3571 \text{ (M.W. = 17,800)}$$

$$K_{av} \text{ of PAI,} \quad \frac{61.95 - 51.1}{291.9 - 51.1} = 0.0450 \text{ (M.W. = 185,000)}$$

$$K_{av} \text{ of PAII,} \quad \frac{134.7 - 51.1}{291.9 - 51.1} = 0.3471 \text{ (M.W. = 18,000)}$$

The molecular weights corresponding to the $K_{av}$ values of SAI, SAII, PAI, and PAII are 220,000, 17,800, 185,000, and 18,000.

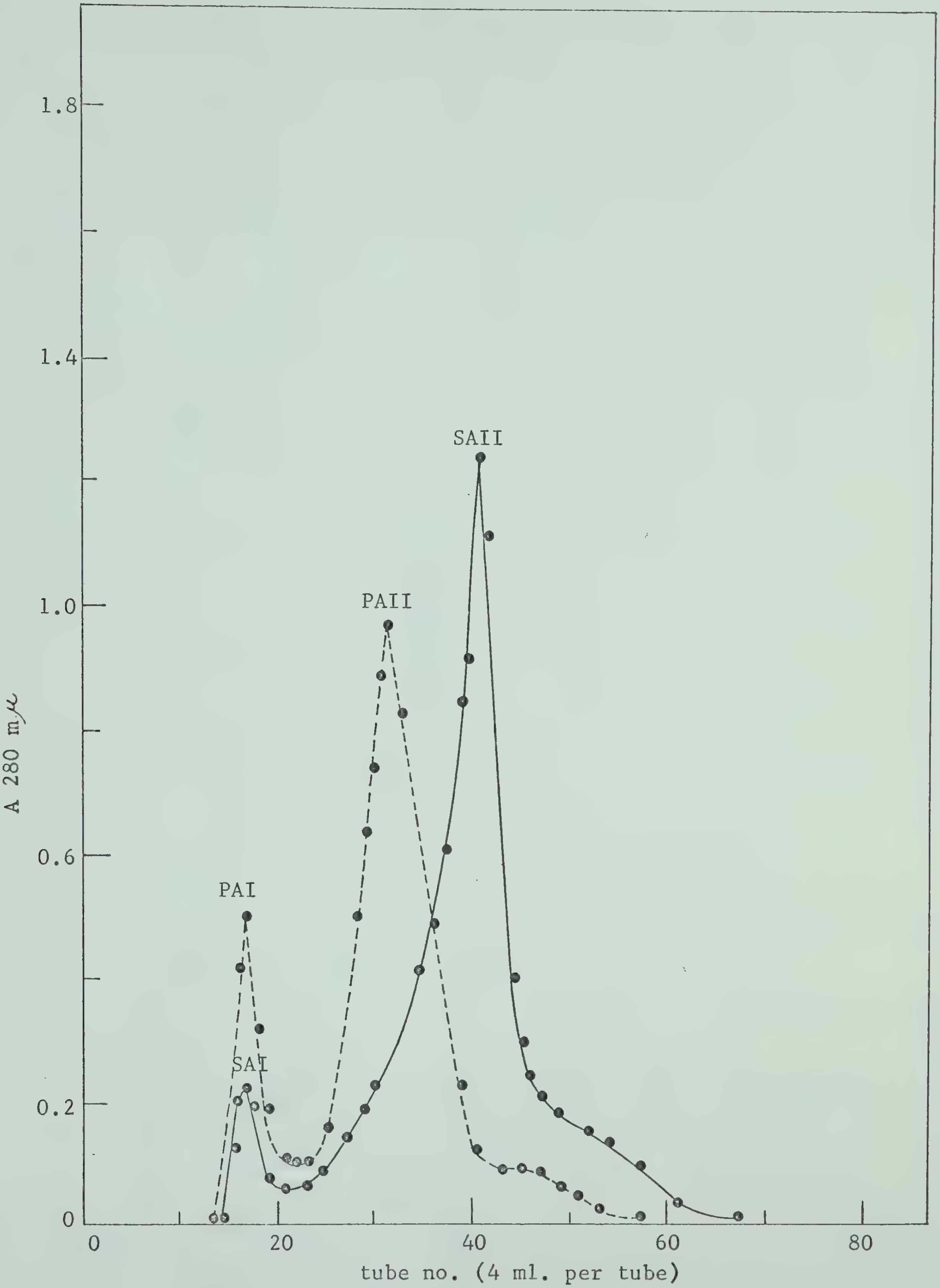


Figure 12. Elution curves on Sephadex G-100 of protein extracted with 0.05 M acetic acid, pH 3.1. Eluant used, acetate buffer pH 4.1.

——— Soluble fractions

----- Insoluble fractions

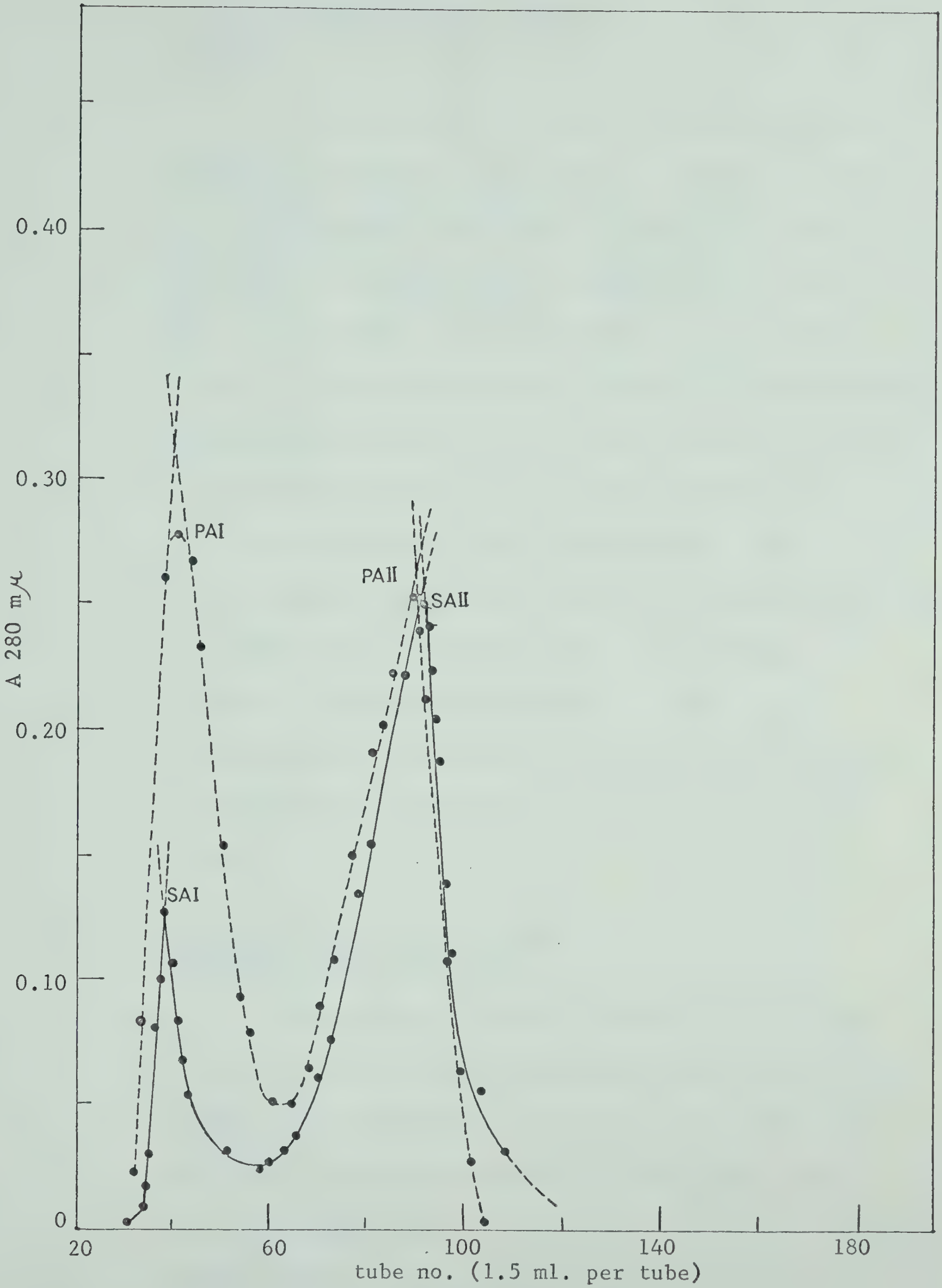


Figure 13. Elution curves on Sephadex G-100 for molecular weight determinations of proteins extracted with 0.05 M acetic acid, pH 3.1. Eluant used, phosphate buffer pH 6.9, containing 0.05 M sodium chloride.

——— Soluble fractions

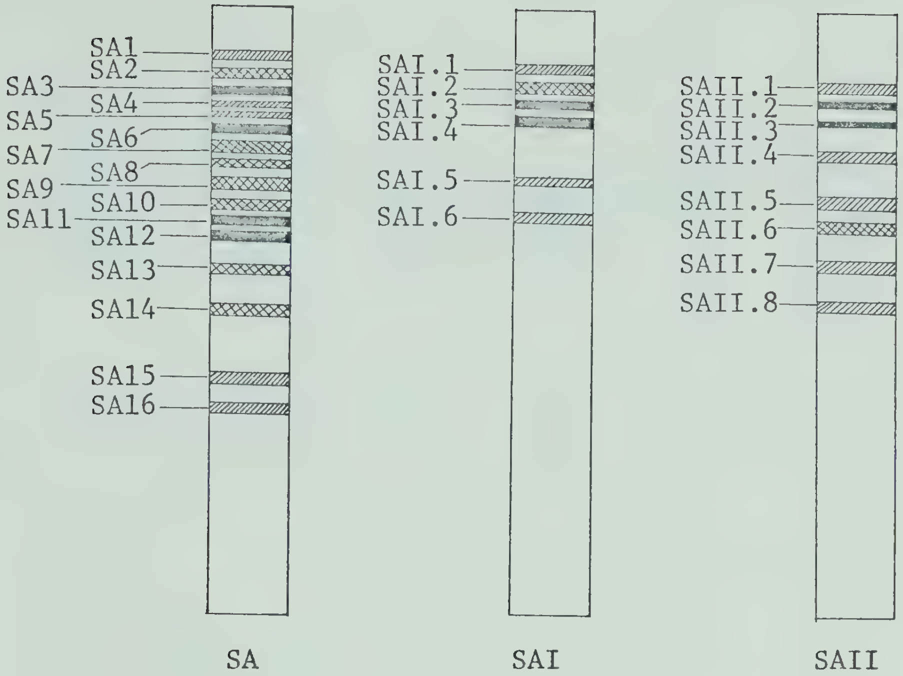
----- Insoluble fractions

### b. Polyacrylamide disc gel electrophoresis of protein extracted with 0.05 M acetic acid.

Protein extracted in acetic acid gave electrophoretic patterns as shown in Figs. 14 and 15. Of the soluble fraction, the gel SAI contains protein band SAI.2 which has the same $R_p$ value as band SAII.1 in gel SAII. The rate of migration of protein SAI.1 is in between protein SA1 and SA2 which were detected in the unfractionated soluble protein, but the similar characteristics in dye absorption seems to suggest that the band SAI.1 may be the same as protein SAI. The bands SA2, SA15 and SA16 were probably lost during the process of fractionation as they were not detected in either of the two gels. The diagram for PAII was made according to the results expected by the comparison of gel PAI to the unfractionated insoluble fraction PA. It consists of five faint bands.

### c. Amino acid analyses of protein extracted with acetic acid.

The amino acid analyses carried out on protein fractions extracted with 0.05 M acetic acid are given in Table 5. In comparison, fraction SAI, SAII, PAI, and PAII show similar distribution of amino acids. The detailed differences are in fraction SAI, which contains higher aspartic acid and lower glutamic acid than the others, fraction SAII which contains high methionine, and fraction PAII which is low in alanine. All fractions, in

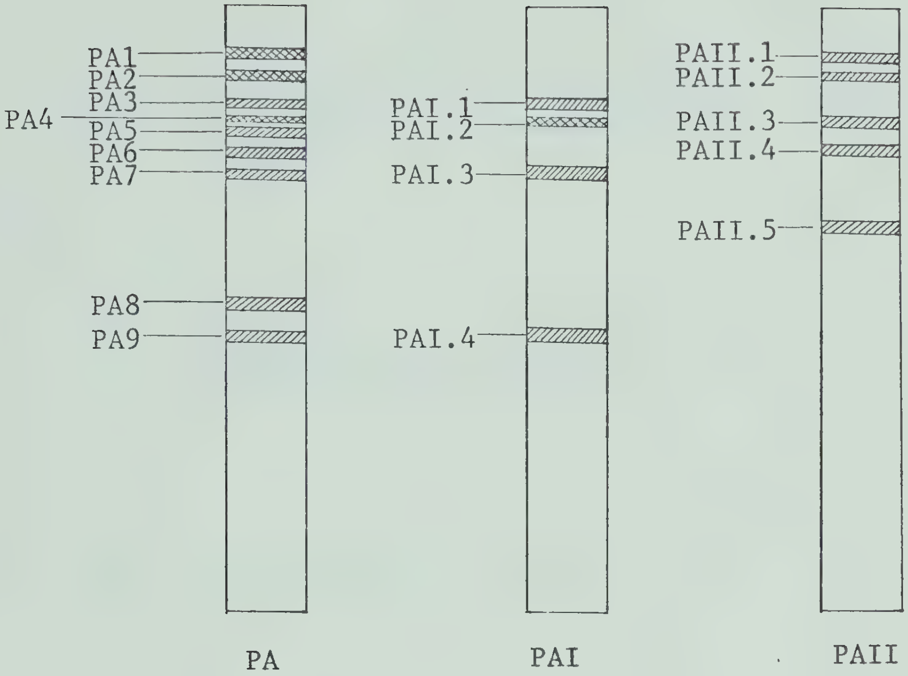


| Bands | $R_p$ |
|---|---|
| SA1 | .1059 |
| SA2 | .1247 |
| SA3 | .1512 |
| SA4 | .1662 |
| SA5 | .1973 |
| SA6 | .2188 |
| SA7 | .2454 |
| SA8 | .2568 |
| SA9 | .2838 |
| SA10 | .3241 |
| SA11 | .3483 |
| SA12 | .3947 |
| SA13 | .4357 |
| SA14 | .5069 |
| SA15 | .6354 |
| SA16 | .6803 |

| Bands | $R_p$ |
|---|---|
| SAI.1 | .1112 |
| SAI.2 | .1504 |
| SAI.3 | .1692 |
| SAI.4 | .2021 |
| SAI.5 | .2884 |
| SAI.6 | .3510 |

| Bands | $R_p$ |
|---|---|
| SAII.1 | .1548 |
| SAII.2 | .1725 |
| SAII.3 | .2290 |
| SAII.4 | .2516 |
| SAII.5 | .3258 |
| SAII.6 | .3870 |
| SAII.7 | .4274 |
| SAII.8 | .5000 |

Figure 14. Schematic diagrams of electrophoretograms of separated soluble fractions of proteins extracted with 0.05 M acetic acid.



| Bands | $R_p$ |
|---|---|
| PA1 | .1097 |
| PA2 | .1455 |
| PA3 | .1810 |
| PA4 | .1973 |
| PA5 | .2225 |
| PA6 | .2551 |
| PA7 | .2863 |
| PA8 | .5014 |
| PA9 | .5489 |

| Bands | $R_p$ |
|---|---|
| PAI.1 | .1810 |
| PAI.2 | .1977 |
| PAI.3 | .2870 |
| PAI.4 | .5474 |

| Bands | $R_p$ |
|---|---|
| PAII.1 | .1144 |
| PAII.2 | .1442 |
| PAII.3 | .2131 |
| PAII.4 | .2507 |
| PAII.5 | .3824 |

Figure 15. Schematic diagrams of electrophoretograms of separated insoluble fractions of proteins extracted with 0.05 M acetic acid.

Table 5.

Amino acid analyses of rapeseed protein extracted with dilute acetic acid (expressed as percent of total amino acid).

| Amino acids | Gel filtration fractions | | | | |
|---|---|---|---|---|---|
| | SAI | SAII | PAI | PAII | AP |
| Aspartic acid | 7.92 | 3.34 | 1.96 | 2.16 | 10.01 |
| Threonine | 5.37 | 3.85 | 3.00 | 3.06 | 6.34 |
| Serine | 6.61 | 4.28 | 4.00 | 4.03 | 5.60 |
| Glutamic acid | 12.52 | 25.43 | 26.90 | 27.98 | 15.15 |
| Proline | 7.51 | 8.93 | 9.96 | 10.04 | trace |
| Glycine | 5.22 | 4.22 | 3.75 | 3.84 | 6.58 |
| Alanine | 4.78 | 4.00 | 3.70 | 0.79 | 7.81 |
| Cystine | 6.14 | 4.69 | 5.00 | 5.20 | trace |
| Valine | 5.63 | 4.76 | 4.49 | 5.15 | 5.45 |
| Methionine | 2.45 | 1.74 | 6.93 | 2.01 | trace |
| Isoleucine | 4.07 | 3.30 | 3.13 | 3.26 | 4.99 |
| Leucine | 7.34 | 6.61 | 6.47 | 6.87 | 7.91 |
| Tyrosine | 2.66 | 1.73 | 1.22 | 1.62 | 11.04 |
| Phenylalanine | 5.85 | 3.35 | 2.84 | 3.31 | 5.82 |
| Lysine | 7.27 | 8.28 | 7.52 | 8.00 | 5.74 |
| Histidine | 2.70 | 4.28 | 3.81 | 3.78 | 2.03 |
| Arginine | 5.86 | 7.13 | 5.24 | 5.82 | 5.46 |

SA Soluble fraction on dialysis of protein extracted with 0.05 M acetic acid.

PA Insoluble fraction on dialysis of protein extracted with 0.05 M acetic acid.

AP Protein extracted with 0.05 M acetic acid after extraction with 0.01 M sodium pyrophosphate.

common, contain high lysine content. Using 0.05 M acetic acid to extract the protein from the residue remaining after using 0.01 M sodium pyrophosphate resulted in only small amount of protein with high aspartic acid, threonine and tyrosine, but slightly low in basic amino acids and decidedly deficient in proline, cystine and methionine.

### (4) Protein extracted with 0.2 percent sodium hydroxide

#### a. Gel filtration on Sephadex and molecular weight determination

The elution curve for the chromatography of protein extracted with 0.2 percent sodium hydroxide (no precipitate formed during dialysis) on Sephadex G-100 shows three separated fractions (Fig. 16). The molecular weight was determined only for fraction hI since the other two fractions were present in only small amounts and the peaks were too broad for precise determination of elution volumes. The elution volume, $K_{av}$ and the molecular weight of hI are 57.9 ml, 0.0282, and 218,000 respectively. The elution pattern of peak hI is shown in Figure 17.

#### b. Polyacrylamide disc gel electrophoresis (Fig. 18)

A thick band was detected in all fractions with somewhat too faint, fast-moving bands in gel hI. Diffusion found below the detected protein bands was

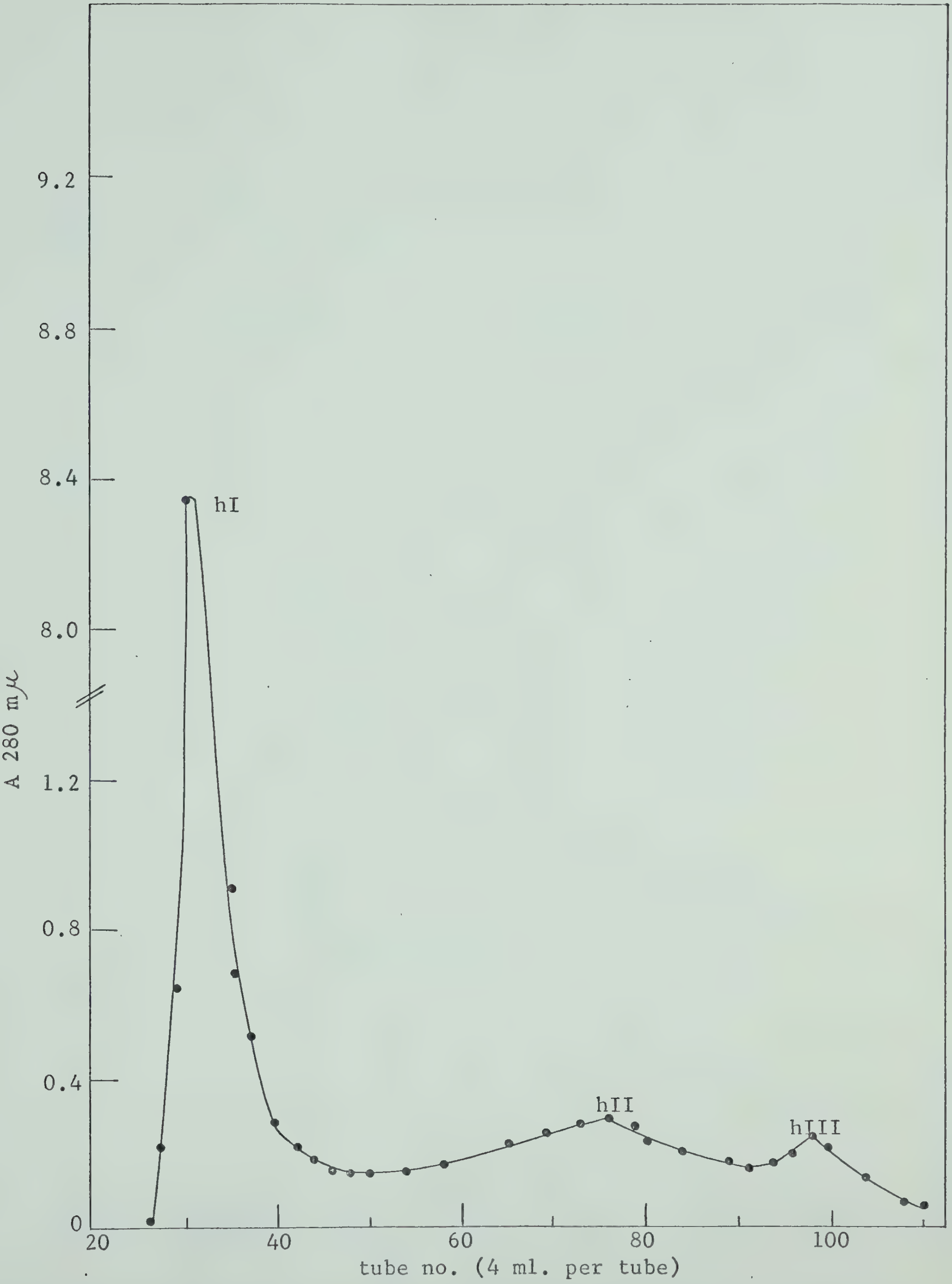


Figure 16. Elution curves on Sephadex G-100 of proteins extracted with 0.2 percent sodium hydroxide. Eluant used, borate buffer pH 9.0.

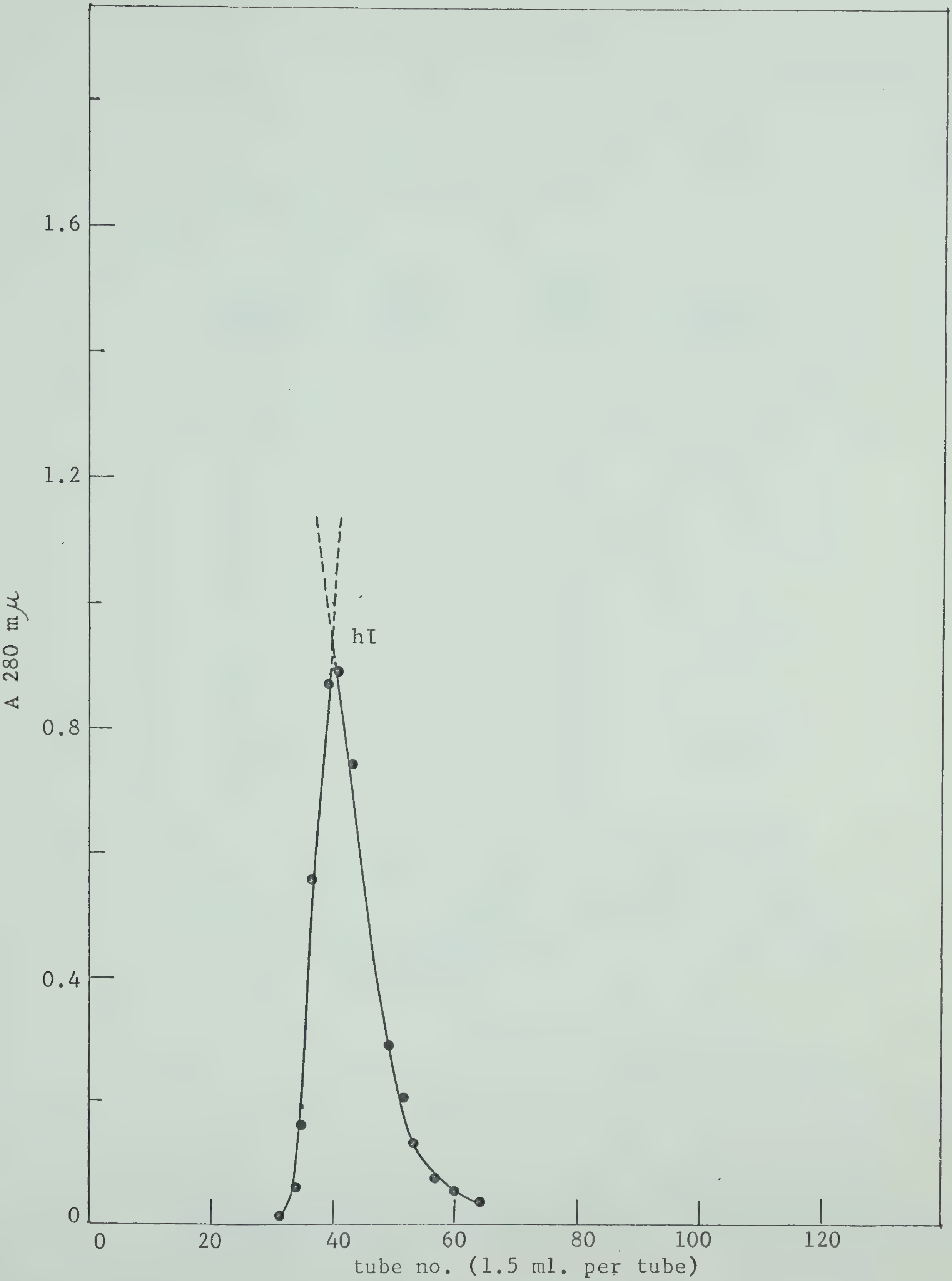
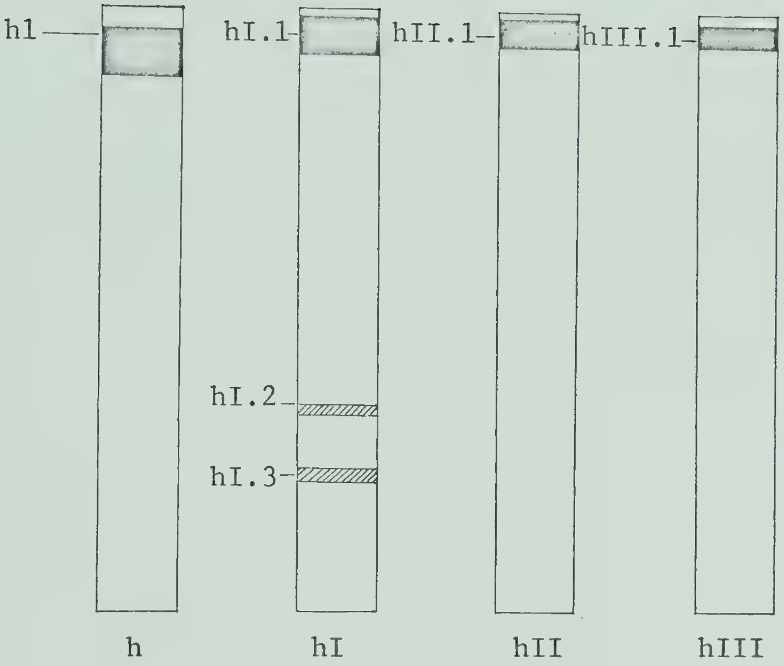


Figure 17. Elution curve on Sephadex G-100 for molecular weight determinations of proteins extracted with 0.2 percent sodium hydroxide. Eluant used, phosphate buffer pH 6.9, containing 0.05 M sodium chloride.



| Band | Rp |
|---|---|
| h1 | .0752 |

| Bands | Rp |
|---|---|
| hI.1 | .0383 |
| hI.2 | .6890 |
| hI.3 | .7827 |

| Band | Rp |
|---|---|
| hII.1 | .0449 |

| Band | Rp |
|---|---|
| hIII.1 | .0420 |

Figure 18. Schematic diagrams of electrophoretograms of separated soluble fractions of proteins extracted with 0.2 percent sodium hydroxide.

probably due to partial hydrolysis by sodium hydroxide, and appeared not only in fraction hI but also in hII and hIII, to a lesser extent.

The amino acid analysis for the sodium hydroxide extract was not performed.

# V. DISCUSSION

## A. Yields of Protein from Different Extracting Solutions

Table 2 shows that 0.2 percent sodium hydroxide extracts more protein than the other aqueous solutions, but it also was found to dissolve more green pigment from the meal. Acetic acid, 0.05 M, is a poor extractant of rapeseed meal protein compared to the other solvents. This is to be expected since the isoelectric point of rapeseed protein is around 4 as reported by Pokorny *et al.* (1964) and higher solubility of most proteins is observed at pH values removed from the isoelectric point. The effectiveness of 10 percent NaCl and 0.01 M sodium pyrophosphate extracting solutions is similar, as indicated by Lowry protein determination, though a difference in specific salt effects was expected, since in addition to disruption of electrostatic forces and hydrogen bonds, salts may especially influence extraction of protein by chelating metal ions. Pyrophosphate buffer has been useful for protein extraction, particularly for enzymes, since many metal-protein complexes are insoluble and the ability of pyrophosphate to chelate metals is of value in bringing these proteins into solution. With the exception of prolamines, which contain a high proportion of nonpolar side chains, most typical albumins or globulins should be soluble in salt solutions at the pH used (pH 7.0). The very small amount of protein obtained from meal re-

extracted with acetic acid after an initial pyrophosphate extraction, indicates that the pyrophosphate salt solution can extract the majority of the protein soluble in acetic acid.

## B. Effects of Dialysis

Extractable protein is reported as two fractions, one soluble after dialysis and the other precipitated on dialysis, quantitated both on a dry weight basis and by Lowry protein determinations (Table 2), but the discussion will emphasize the results of the Lowry method as this is probably more accurate. Dialysis can employ a variety of solvents, but for peptides and proteins aqueous solutions are usually the ones of choice. Distilled water as a solvent nearly always provides a more rapid rate of dialysis than any mixture of solvent or solute, and is preferred unless pH control is required in order to maintain the dialyzing solute in a stable condition, avoiding tendencies of some proteins to dimerize at the pH of distilled water. Since globulin is less soluble in water, dialysis against distilled water was carried out as a preliminary fractionation step to separate this protein from the sodium chloride extract. About 63 percent of the protein soluble in 10 percent sodium chloride is recovered as an insoluble fraction on dialysis.

It was noticed that precipitation by dialysis reduced the solubility of the protein being redissolved in the original extracting solvent. In order to minimize this reduction, the dialysis of the extracts of pyrophosphate, acetic acid and sodium hydroxide was carried out in solutions buffered at pH values close

to the corresponding extraction media, but there was still an amount of protein forming a precipitate at the experimental conditions employed. The results obtained during dialysis of protein extracted with 0.01 M sodium pyrophosphate against borate buffer, pH 8.6, do not agree with that of Bhatty _et al_. (1968) who reported that no precipitate formed after dialysis. This difference may be due to the concentration differences of the protein in the solution and to the longer dialyzing period of the present study. The amount of the precipitate increases during the prolongation of dialysis. The precipitate from this extract accounted for 31 percent of the total protein extracted. Half of the extracted protein is precipitated during dialysis of the acetic acid extract against 0.005 M acetate buffer. There is no precipitate formed in the aliquot of sodium hydroxide soluble protein, probably because of the hydrolysis to polypeptides. The multiple operations of dialysis, fractionation on a Sephadex G-100 column, and lyophylization resulted in some losses of material. Higher losses in protein of low molecular weight from pyrophosphate and sodium chloride extracts may have been the result of the dialysis operation, wherein low molecular weight particles could pass through the cellophane tubing.

## C. Sephadex Chromatography of Rapeseed Proteins

The molecular weights determined by gel filtration suggest that the fraction designated PPI (precipitated on dialysis) in the chromatogram of protein extracted with 0.01 M sodium pyrophosphate (Fig. 4 and 5) is similar to the fraction designated SPI, the

major constituent of protein soluble in this salt solution. These two fractions, SPI and PPI, have molecular weights in the same range as PSI, the water insoluble protein fraction present in the sodium chloride extract, which has a molecular weight about 170,000 (Fig. 8 and 9). With regard to their solubility and molecular size, the proteins of these fractions are considered to be seed globulins. Neutral salt solutions appear to dissolve low molecular weight proteins and peptides which are eluted late from the column in fractions SPIV, SPV and PSIII. Soluble proteins from fractions SPII, SPIII and SSII, with an average molecular weight of 32,000 (SPII) and 19,000 (SPIII and SSII) are suspected to be albumins. Although the solubility behavior of SSI indicates that it should be classified as an albumin, the molecular weight is much larger than the albumin type, hence, it may be some globulin contaminant resulting from incomplete precipitation during dialysis, even though the molecular weight is less than those of fractions SPI, PPI and PSI. The insoluble material of fraction PPII and PSII are probably precipitates formed by a denaturing process or by an interaction of proteins, since histones often precipitate other proteins from solution. Extraction of proteins with 0.05 M acetic acid resulted in a distribution of protein, quite different from that of the saline dispersible proteins.

The proteins extracted with acetic acid contained none of the low molecular weight proteins found in the neutral salt solutions (Figs. 12 and 13). The protein extracted by acetic acid is largely of molecular weight 17,800 to 18,000, similar to that

of soluble fraction SAII and the insoluble fraction PAII. The lesser fraction resembles the proteins of molecular weight 220,000 and 185,000 of the soluble fraction SAI and insoluble fraction PAI. The SAII and PAII components are probably histones, the basic proteins which are characterized by being soluble in dilute acid solution, while the class to which the high molecular weight protein should belong is not known. The elution patterns in Figure 13 and 14 indicate that the fraction SAII is less soluble in phosphate buffer, pH 6.9, than in acetate buffer, pH 4.1. The proteins designated hI, found in the NaOH extract are as large as that of SAI and show high optical density in the ultraviolet range.

It appears that fractions SPIII, PPII, SSII, SAII and PAII contain the histones of group 2 or group 3, based on the molecular size classification of Murray (1964) and the solubility behavior. These fractions seem to be similar to the 1.7 S protein which Bhatty *et al*. (1968) found in rapeseed *B*. *napus*, Var. Nugget, a basic protein of molecular weight 13,800 ± 300.

## D. Polyacrylamide Gel Electrophoresis

Results of polyacrylamide gel electrophoresis of rapeseed protein, both the unfractionated soluble and insoluble proteins extracted in the four solvent systems (illustrated in Fig. 3) showed that extraction of the meal with neutral salt solutions and 0.05 M acetic acid yields a mixture slow- and fast-moving components, whereas extraction with 0.2 percent sodium hydroxide yields a single protein component which remains almost at the

origin of small pore gel. The most heterogeneity seems to be present in the 10 percent sodium chloride extract which reveals 21 components in the soluble fraction (SS) and 16 components in the insoluble fraction (PS). Most of the bands of the insoluble fractions are detected at positions corresponding to those of several soluble protein bands, indicating that there is some cross contamination of the protein constituents from each system. The electrophoretic patterns of neutral salt-soluble proteins of *B. campestris* show more complexity than those observed by Vaughan *et al*. (1966). These results may be due in part to the better staining affinity of Coomassie Brilliant Blue R250 over Amido Black, as stated by Chrambach *et al*. (1967), and also to the fact that urea is a strong dissociating agent. Because of the use of urea in the gels, some bands may be attributed to protein complexes at various stages of association and dissociation. It is expected that DTT in the gels will have a similar effect.

Zone electrophoretic patterns of rapeseed protein extracted with pyrophosphate (as shown in Fig. 6) indicates the largest amount of heterogeneity in the proteins of fraction SPI. Fractions from the pyrophosphate extract contain much more of the very slow moving components than those of 10 percent sodium chloride and acetic acid extracts. Comparison of the globulins (fractions SPI, PPI, SSI and PSI, shown in Figs. 6, 7, 10, and 11 respectively) for the two salt solutions examined shows that they contain a similar number of bands and are characterized by approximately the same $R_p$ values. The majority of proteins observed in the insoluble fractions PPI and PPII (Fig. 7) are slow

moving bands, while the insoluble fractions PSI and PSII (Fig. 10) contained both fast- and slow-moving protein bands. Proteins PPII.1 and PPII.2 resemble PSII.1 and PSII.2, respectively, in their rate of migration and staining characteristics. The low molecular weight proteins of the two salt solutions show different electrophoretic behavior in the alkaline gels employed in the study.

Although acetic acid can extract only a small amount of protein from rapeseed meal, gel electrophoresis shows the presence of a number of bands (shown in Figs. 14 and 15). Some of proteins which could not be detected after fractionation on Sephadex G-100 were probably lost during the many steps of sample preparation or possibly as an effect of the dissociating agents. At least 8 bands were present in the histone fraction, SAII, and these bands have migration characteristics similar to several protein bands of the SSII fraction of the NaCl extract.

The gel patterns of protein extracted into 0.2 percent sodium chloride are distinct from those soluble in the other extractants. They reveal a single band in all fractions which migrates very slowly towards the cathode as a thick zone. The diffusion effects observed in the staining of these proteins, suggested that they are relatively low molecular weight molecules. Perhaps they resulted from the alkaline hydrolysis of some of the protein to polypeptide which would account for their ready diffusion from the gels.

In many gels there are some components which migrate within the large pore gel but do not enter the smaller pore gel

layer. This phenomenon may be partly attributed to their large molecular size and partly to their neutral or strongly basic character. The number of protein bands in the various solubility groups within each extracting medium are widely varied, and it is impossible to correlate the bands of the electrophoretograms with specific rapeseed proteins. Interpretation is further complicated by the dissociating effect of the urea and DTT employed in this study. Moreover, as shown by disc electrophoresis, there was a considerable overlapping between neighboring fractions since the peaks in the chromatograms do not go to the base line and therefore add to the complexity of the results obtained.

### E. Amino Acid Analyses of Fractionated Rapeseed Protein

Amino acid analyses of the gel filtration fractions SPI and PPI (reported in Table 3), which are considered to be protein globulins, contain uniformly high aspartic acid, methionine, leucine, arginine and tyrosine residues, but they are low in their cystine and histidine content. Fraction SPII and SPIII closely resemble each other in amino acid composition, but again the valine content is different. Fraction SPIV of the low molecular weight proteins is low in aspartic acid, methionine and tyrosine, but contains high levels of the basic amino acids, while fraction SPV is high in serine, glutamic acid, proline and glycine but contains only trace amounts of the basic amino acids and suffers from the complete absence of cystine. The strangely low values in

all basic amino acids of SPV may be due to an error in the operation of the chromatographic column. All fractions of the pyrophosphate extract appear to be good sources of the essential amino acids threonine, leucine, isoleucine and phenylalanine.

As is apparent from the data in Table 4, the similarity in the amino acid compositions of fractions SSI and PSI to those of SPI and PPI support the results obtained from the solubility, gel filtration and electrophoresis behavior indicating that these proteins belong to the same group. Fraction SSII is different from SSI and SSIII in amino acid content, even though this fraction must be heavily contaminated with the latter fractions. SSII is lower in aspartic acid, but richer in proline and cystine than SSI and SSIII. PSII shows high levels of glutamic acid, proline and cystine whereas tyrosine, lysine, histidine and arginine are low. Generally, the essential amino acid contents of the protein from the 10 percent sodium chloride solution seem comparable to the other two solvent systems except that a rather low content of lysine and histidine is shown in the fraction insoluble on dialysis (PS).

The amino acid composition of the fractionated proteins from 0.05 M acetic acid extract (listed in Table 5) shows that all contain large amounts of the basic amino acids, as well as proline and considerable quantities of cystine; although all are low in tyrosine. The significantly high lysine, histidine, arginine and proline contents strongly support the contention that these are histone like proteins, suggested by the solubility and molecular weight determinations. The electrophoresis patterns do not give

much information in this regard, other than indicating the complexity of the protein mixture.

It is important to note that fractions SSI, PSI and SAI contain some quantity of carbohydrate. This assumption is supported by the occurrence of a black suspension in the hydrolyzates. The presence of carbohydrates in these fractions may result in negligible to large losses of amino acid during hydrolysis and this therefore may be responsible for some changes in the amino acid patterns of these fractions.

This preliminary investigation on whole rapeseed meal proteins suggests that similar commercial techniques can be developed to purify the proteins. It is apparent from our data that approximately 50 percent of the total protein can be extracted with salt solutions, and dialyzed free from toxic substances of low molecular weight. The protein might then be used in a manner similar to any other protein supplement. However, in order to make the rapeseed proteins more suitable as a food or as an ingredient of foods, the basic aspects of processing need more extensive studies. Dialysis appears useful as a method for a selective separation of those proteins of high nutritional value, however, losses through the dialysis tubing of low molecular weight proteinaceous materials may have been large and hence these losses must be considered. Information obtained from detailed studies on the characteristics of rapeseed protein using different analytical methods may add some knowledge to the chemistry of rapeseed proteins and be at least indirectly valuable to industrial research, in the same way as the ever-expanding knowledge of the

chemistry and properties of soybean proteins increase their utilization in new developments in processing technology.

# VI. CONCLUSIONS

1. Sodium hydroxide, 0.2 percent, was the most effective solvent employed, giving the highest yield of proteinaceous material, but this solvent appears to cause an alteration of proteins which makes further gel electrophoresis study and amino acid analyses unreliable. Although the two neutral salt solutions yield a comparable amount of protein, they have different efficiency in their extracting power on the various classes of proteins. Gel electrophoretic patterns of the NaCl extract reveals this solvent has the most heterogeneity in the protein components. The overall essential amino acid contents are quite similar for neutral salt soluble proteins.
2. Gel filtration, based on the molecular sieving effect, fractionates the rapeseed protein which is soluble in each of the four solvent systems employed into three main groups. They are the proteins of molecular weights of between 160,000 to 220,000 intermediate molecular weights in the range of 32,000 to 19,000 and the low molecular weight proteins of less than 10,000.
3. Seed globulins which are soluble in neutral salt solutions are present in the largest amount and are well characterized in the study. They are high in aspartic acid, methionine, leucine, arginine and tyrosine residues, but low in cystine and histidine. Neutral saline solutions also dissolve low molecular weight

proteins and peptides which are neither precipitated nor dialyzable during the operation in cellophane tubing. These low molecular weight proteins are absent in the acetic acid extract.

4. The major group of proteins which are soluble in 0.05 M acetic acid solution are characterized by having an average molecular weight of about 17,000 to 19,000 and contain high levels of the basic amino acids, as well as proline and cystine. These proteins appear to be histones.
5. The amino acid composition of the fractions separable during gel filtration are quite different from each other, but the essential amino acid contents obtained support the idea that rapeseed is a good source of nutritious protein.
6. Electrophoresis on polyacrylamide gels is an efficient technique for analysis of rapeseed proteins. It demonstrates the heterogeneity in most of apparently homogeneous fractions from the Sephadex G-100 columns. At present the interpretation of electrophoretic characteristics is somewhat complicated due to cross-contamination between the adjacent fractions and the dissociating effect of the 8M urea-DTT solvent, but it appears that the low molecular weight proteins are more heterogeneous than those of high molecular weight.
7. The Standard Kit curve is not linear at the lower end of the molecular weight scale for Sephadex G-100. Since molecular weight estimations by gel filtration involve in principle a comparison of the behavior of the compounds under investigation with that of related weight and gel filtration behavior, the choice of proteins as molecular weight standards can considerably influence the

results of molecular weight estimations.

# VII. REFERENCES

Ågren, G. 1952. Microbiological determination of amino acids in foods. Acta Chem. Scand. 6: 608-609.

Andrews, P. 1962. Estimation of molecular weights of proteins by gel filtration. Nature 196: 36-39.

Appelqvist, L.-Å. 1962. Hereditary variation in content of isothiocyanates and thiooxazolidinethiones in seeds of rape and turnip rape. Acta Chem. Scand. 16: 1284-1285.

Astwood, E. B., M. A. Greer, and M. C. Ettlinger. 1949. 1-5-vinyl-2-thiooxazolidone, an antithyroid compound from yellow turnip and from brassica seeds. J. Biol. Chem. 181: 121-130.

Ballester, D., R. Rodrigo, J. Nakouzi, C. O. Chichester, E. Yáñez, and F. Mönckeberg. 1970a. Rapeseed meal II. Chemical composition and biological quality of the protein. J. Sci. Fd Agric. 21: 140-142.

Ballester, D., R. Rodrigo, J. Nakouzi, C. O. Chichester, E. Yáñez, and F. Mönckeberg. 1970b. Rapeseed meal III. A simple method for detoxification. J. Sci. Fd Agric. 21: 143-144.

Baptist, N. G. 1963. Free amino acids in the endosperm of the developing coconut (Cocos nucifers). J. Exptl. Bot. 14: 29-41.

Beare, J. S., J. A. Campbell, C. G. Youngs, and B. M. Craig. 1963. Effects of saturated fat in rats fed rapeseed oil. Can. J. Biochem. and Physiol. 41: 605-612.

Bell, J. M. 1955. The nutritional value of rapeseed oilmeal -- a review. Can. J. Agric. Sci. 35: 242-251.

Bell, J. M. 1957. Growth depressing factors in rapeseed oilmeal III. Studies on counteraction by steam processing, extraction and dietary supplements. Can. J. Animal Sci. 37: 31-42.

Bell, J. M., and E. Baker. 1957. Growth depressing factors in rapeseed oil meal II. Studies on counteraction by dietary supplements. Can. J. Animal Sci. 37: 21-30.

Bell, J. M., and R. J. Belzile. 1965. Goitrogenic properies (of rapeseed meal). p. 45-60 in "Rapeseed Meal for Livestock and Poultry -- a Review." Can. Dept. Agr. Pub. 1257, Ottawa, Canada.

Bell, J. M., R. K. Downey, and L. R. Wetter. 1967. Oil and meal from Canadian rapeseed. Can. Dept. Agr. Pub. 1183, 20p.

Belzile, R., J. M. Bell, and L. R. Wetter. 1963. Growth depressing factors in rapeseed oil meal V. The effects of myrosinase activity on the toxicity of the meal. Can. J. Animal Sci. 43: 169-173.

Bensabat, L., and V. L. Frampton. 1958. Effect of processing on the epsilon-amino groups of lysine in peanut proteins. J. Agric. Food Chem. 6: 778.

Bhatty, R. S., S. L. Mckenzie, and A. J. Finlayson. 1968. The proteins of rapeseed (B. napus L.) soluble in salt solution. Can. J. Biochem. 46: 1191-1197.

Boulter, D., D. A. Therman, and E. Derbyshire. 1968. A disc electrophoretic study of globulin proteins of legume seeds with reference to their systematics. New Phytol. 66: 27-56.

Booth, M. R. 1970. Isolation of further components from wheat gliadins. J. Sci. Fd Agric. 21: 185-186.

Booth, M. R., and J. A. D. Ewart. 1970. Relationship between wheat proteins. J. Sci. Fd Agric. 21: 187-193.

Briggs, D. R., and W. J. Wolf. 1957. Studies on the cold-insoluble fraction of the water-extractable soybean proteins I. Polymerization of the 11 S component through reactions of sulfhydryl groups to form disulphide bonds. Arch. Biochem. and Biophys. 72: 127-144.

Brohut, S., and C. Sandegren. 1954. Seed proteins. in "The Proteins." 2A, p. 287-512. Neurath, H., and Bailey, K., eds. Academic Press, New York.

Carter, F. L., V. O. Cirino, and L. E. Allen. 1961. Effect of processing on the composition of sesame seed oil meal. J. Ame. Oil Chem. Soc. 39: 148-150.

Catsimpoolas, N., and C. Ekenstam. 1969. Isolation of alpha, beta, and gamma conglycinins. Arch. Biochem. and Biophys. 129: 490-497.

Chandrasekaran, A., and K. W. King. 1967. Enzymatic modification of the extractability of protein from coconut (Cocos nucifera). J. Agric. Fd Chem. 15: 305-309.

Chrambach, A., R. A. Reisfield, M. Wyckoff, and J. Jaceari. 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. Anal. Biochem. 20: 150-154.

Chelliah, J., and N. G. Baptist. 1969. Extraction of protein from expeller- and solvent-extracted coconut meat by dilute acid, alkali, and salt solutions. J. Sci. Food Agric. 20: 49-53.

Chisholm, M. D., and L. R. Wetter. 1964. Biosynthesis of mustard oil glucosides IV. The administration of methionine - $C^{14}$ and related compounds to horseradish. Can. J. Biochem. 42: 1033-1040.

Chisholm, M. D., and L. R. Wetter. 1967. The biosynthesis of some isothiocyanates and oxazolidinethiones in rape (Brassica campestris L.). Pl. Physiol. 42: 1726-1730.

Clandinin, D. R., and L. Baylay. 1963. Effect of variety and growing conditions on the protein and amino acid composition of rapeseed. Can. J. Animal Sci. 43: 65-67.

Clandinin, D. R., R. Renner, and A. R. Robblee. 1959. Rapeseed oil meal studies I. Effects of variety of rapeseed, growing environment and processing temperatures on the nutritive value and chemical composition of rapeseed oil meal. Poultry Sci. 38: 1367-1372.

Clandinin, D. R., and E. W. Tajcnar. 1961. Rapeseed oil meal studies III. Effects of variations in cooking and conditioning temperatures used during processing of rapeseed oil meals. Poultry Sci. 40: 291-298.

Conkerton, E. J., and V. L. Frampton. 1959. Reaction of gossypol with free $\epsilon$-amino groups of lysine in proteins. Arch. Biochem. and Biophys. 81: 130-134.

Conkerton, E. J., W. H. Martinez, G. E. Mannand, and V. L. Frampton. 1957. Changes induced by autoclaving a solvent-extracted cottonseed meal. J. Agric. Food Chem. 5: 460-463.

Craig, B. M. 1961. Varietal and environmental effects on rapeseed III. Fatty acid composition of 1958 varietal tests. Can. J. Plant Sci., 41: 204-210.

Craig, B. M., and L. R. Wetter. 1959. Varietal and environmental effects on rapeseed II. Fatty acid composition of the oil. Can. J. Plant Sci. 39: 437-442.

Crow, M. J. A., and J. A. Rothfus. 1968. Chromatography of proteins from wheat gluten on polyacrylamide gel. Cereal Chem. 45: 413-420.

Danielson, C. E. 1956. Plant proteins. Ann. Rev. Plant Physiol. 7: 215-236.

Davis, B. J. 1964. Disc electrophoresis II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. V.121, Art.2. p. 404-427.

Dechary, J. M., K. F. Talluto, W. J. Evans, W. B. Carney, and A. M. Altschul. 1961. α-Conarachin. Nature 190: 1125-1126.

Determann, H., and W. Michel. 1966. The correlation between molecular weight and elution behavior in the gel chromatography of proteins. J. Chromatog. 25: 303-313.

Dieckert, J. W., J. E. Snowden, Jr., A. T. Moore, D. C. Heinzelman, and A. M. Altschul. 1962. Composition of some subcellular fractions from seeds of Arachis hypogaea. J. Fd Sci. 27: 321-325.

Dimler, A. J. 1969. Oilseed proteins. Chem. Eng. Prog. 65: 20-26.

Downey, R. K., B. M. Craig, and C. G. Youngs. 1969. Symposium: Modification of oilseeds through plant breeding. J. Amer Oil Chem. Soc. 46: 121-123.

Downey, R. K., and B. L. Harvey. 1963. Methods of breeding of oil quality in rape. Can. J. Plant Sci. 43: 271-275.

Eapen, K. E., N. W. Tape, and R. P. A. Sims. 1968. New process for the production of better quality rapeseed oil and meal I. Effect of heat treatments on enzyme destruction and color of rapeseed oil. J. Amer. Oil Chem. Soc. 45: 194-196.

Eapen, K. E., N. W. Tape, and R. P. A. Sims. 1969. New process for the production of better quality rapeseed oil and meal II. Detoxification and dehulling of rapeseed-feasibility. J. Amer. Oil Chem. Soc. 46: 52-55.

Eldridge, A. C., and N. J. Wolf. 1967. Purification of the 11 S component of soybean protein. Cereal Chem. 44: 645-652.

Evan, R. J., and H. A. Butts. 1948. Studies on the heat inactivation of lysine on soy bean oil meal. J. Biol. Chem. 175: 15-28.

Evans, W. J., W. B. Carney, J. M. Dechary, and A. M. Altschul. 1962. Zone electrophoresis of conarachin, α-chonarachin and bovine serum albumin on polyacrylamide gel. Arch. Biochem. and Biophys. 96: 233-239.

Finlayson, A. J. 1965. Amino acid recoveries in the analyses of some feed samples. Can. J. Plant Sci. 45: 184-188.

Finlayson, A. J. 1966. Incorporation of $^{14}$C into two proteins from rapeseed. (Brassica napus L.). Can. J. Biochem. 44: 997-1004.

Finlayson, A. J. 1967. Changes in the aqueous ammonia-soluble proteins of rapeseed, (Brassica napus L.) during the maturing period. Can. J. Biochem. 45: 1225-1231.

Goering, K. J., O. O. Thomas, D. R. Beardsley, and W. A. Eurran, Jr. 1960. Nutritional value of mustard and rapeseed meals as protein source for rats. J. Nutrition 72: 210-216.

Granath, K. A., and P. Flodin. 1961. Fractionation of dextran by the gel filtration method. Makromol. Chem. 48: 160-171.

Jackson, N. 1969. Toxicity of rapeseed meal and its use as a protein supplement in the diet of two hybrid strains of caged laying hens. J. Sci. Fd Agric. 20: 734-740.

Jackson, P., J. M. Milton, and D. Boulter. 1967. Fingerprint patterns of the globulin fraction obtained from seeds of various species of Fabaceae. New Phytol. 66: 47-56.

Johns, C. O., and D. B. Jones. 1916. The proteins of the peanut, Arachis hypogaea. I. The globulins arachin and conarachin. J. Biol. Chem. 28: 77-87.

Johnson, P., and E. M. Shooter. 1950. The globulins of the ground nut (Arachis hypogaea). I. Investigation of arachin as a dissociation system. Biochim. et Biophys. Acta. 5: 361-375.

Jone, B. B., and F. A. Csonka. 1925. Proteins of the cottonseed. J. Biol. Chem. 64: 673-683.

Josefsson, E. 1970. Glucosinolate content and amino acid composition of rapeseed (B. napus) meal as affected by sulfur and nitrogen nutrition. J. Sci. Fd Agric. 21: 98-102.

Joubert, F. J. 1955. Sunflower seed proteins. Biochim. et Biophys. Acta. 16: 520-523.

Kaye, R., L. A. Barness, A. Valyasevi, and J. Knapp. 1961. Nitrogen balance studies of plant proteins in infant in "Progress in Meeting Protein Needs in Infants and Preschool Children." Natl. Acad. Sci. -Natl. Res. Council, Publ. 843, p.297-312. Washington, D.C.

Klain, G. J., D. C. Hill, H. D. Branion, and J. A. Gray. 1956. The value of rapeseed oil meal and sunflower seed oil meal in chick starter rations. Poultry Sci. 35: 1315-1326.

Koshiyama, I. 1968a. Chromatographic and sedimentation behavior of a purified 7 S protein in soybean globulin. Cereal Chem. 45: 405-412.

Koshiyama, I. 1968b. Chemical and physical properties of a 7 S protein in soybean globulins. Cereal Chem. 45: 394-403.

Kratzer, F. H., P. N. Davis, D. E. Williams, and B. J. Marshall. 1954. Factors influencing the growth of chicks and poults fed rations containing rapeseed oil meal. J. Nutrition. 53: 407-418.

Krober, O. A., and S. J. Gibbons. 1962. Nonprotein nitrogen in soybeans. J. Agric. Food Chem. 10: 57-59.

Lathe, G. H., and C. R. J. Ruthven. 1956. The separation of substances and estimation of their relative molecular sizes by the use of columns of starch in water. Biochem. J. 62: 665-674.

Laurent, T. C., and J. Killander. 1963. A theory of gel filtration and its experimental varification. J. Chromatog. 14: 317-330.

Lea, C. H., and R. S. Hannan. 1950. Studies of the reaction between proteins and reducing sugars in the 'Dry' state II. Further observations on the formation of the casein-glucose complex. Biochim. et Biophys. Acta. 4: 518-531.

Manns, I. G., and J. B. Bowland. 1963. Solvent-extracted rapeseed oil meal as a protein source for pigs and rats. Can. J. Animal Sci. 43: 252-263.

Martinez, W. H., and V. L. Frampton. 1961. Effect of gossypol and raffinose on lysine content and nutritive quality of proteins in meals from glandless cottonseed. J. Agric. Food Chem. 9: 64-66.

Martinez, W. H., and V. L. Frampton. 1962. Isolation and chromatographic characterisation of low molecular weight cottonseed protein. J. Agric. Food Chem. 10: 412-415.

Matét, J., R. Nentagne and A. Buchy. 1949. Oléagineux 4: 145. (Cited by L. R. Wetter, 1965, Can. Dept. Agric. Pub. 1257, p. 32).

McGhee, J. E., L. D. Kirk, and G. C. Mustakas. (1964). Mustard seed processing: simple methods for following heat damage to protein meals. J. Amer.Oil Chem. Soc. 41: 359-362.

Milner, M. (1966). General outlook for seed protein concentrates in "World Protein Resources." Advances in Chemistry Series 57. Robert, F. G., ed., p. 52-64. American Chemical Society, Washington, D.C.

Miller, R. W., C. H. Van Etten, C. McGrew, and I. A. Wolff. 1962. Amino acid composition of seed meals from forty-one species of cruciferae. J. Agric. Food Chem. 10: 426-430.

Mitzuno, T. 1958. Nippon Nogei Kagabu Kaish. 32: 340. (Cited by L. K. Wetter, 1965, Can. Dept. Agric. Pub. 1257, p. 32).

Murray, K. 1963. The heterogeneity of histones in "The Nucleohistones". Bonner, J., and P. Ts'O, eds., p. 21-35. Holden-Day Inc., San Francisco.

Mustakas, G. C., L. D. Kirk, and E. L. Griffin, Jr. 1962. Mustard seed processing: bland protein meal, bland oil, and allyl isothiocyanate as a by-product. J. Amer. Oil Chem. Soc. 39: 372-377.

Mustakas, G. C., L. D. Kirk, V. E. Sohns, and E. L. Griffin, Jr. 1965. Mustard seed processing: improved method for isolating the pungent factor and controlling protein quality. J. Amer. Oil Chem. Soc. 42: 33-37.

Naismith, W. E. F. 1955. Ultracentrifuge studies on soya bean protein. Biochim. et Biophys. Acta. 16: 203-210.

Osborne, T. B. 1919. "The Vegetable Proteins", 99p., Longmans, Green and Co.: 39 Paternoster Fow, London.

Osborne, T. B., and G. F. Campbell. 1898. Proteins of the soybean (Glycine hispida). J. Amer. Chem. Soc. 20: 419-428.

Parpia, H. A. B., and N. Subramanian. 1966. Plant protein foods in India, in "Plant Protein Resources". Advances in Chemistry Series 57. Robery, F. G., Ed., p. 112-132. American Chemical Society, Washington, D.C.

Pokorny, J., Z. Sofr, and M. Vodicka. 1964. Protein concentrates from extracted rape seed cake. J. Sci. Fd Agric. Abs. ii-217.

Rachis, J. J., A. K. Smith, and G. E. Babcock, and H. A. Sesame. 1957. An ultracentrifugal study on the association-dissociation of glycinin in acid solution. J. Amer. Chem. Soc. 79: 4655-4658.

Renner, R., D. R. Clandinin, A. B. Merison, and A. R. Robblee. 1953. The effects of processing temperatures on the amino acid content of sunflower seed oil meal. J. Nutrition. 50: 487-490.

Roberts, R. C., and D. R. Briggs. 1965. Isolation and characterization of the 7 S component of soybean globulins. Cereal Chem. 42: 71-85.

Roche, J., and R. Michel. 1946. Sur la valeur alimentaire des proteins des tourteau. Oleagineaux. 1: 205-207. (Cited by J. M. Bell, 1955, Can J. Agric. Sci. 35: 242-251).

Rossi-Fanelli, A., D. Cavallini, B. Mondovi, A. M. Wolf, A. Scioscia-Santoro, and F. Riva. 1965. Studies on cottonseed enzymes I. proteolytic and glutamic dehydrogenase activities in cottonseed. Arch. Biochem. and Biophys. 110: 85-90.

Sallan, H. R. 1964. Factors affecting the composition of Canadian oil seeds. J. Amer. Oil Chem. Soc. 41: 215-218.

Sallans, H. R., K. L. Phillips, J. F. T. Spencer, C. G. Youngs, P. S. S. Dawson, and G. D. Sinclair. 1967. Oil seeds. Praire Regional Laboratory, NRC Review. p. 57-58.

Sjogren, B., and R. Spychalski. 1930. The molecular weight of cocosin. J. Amer. Chem. Soc. 52: 4000-4404.

Smith, A. K., and J. J. Rachis. 1957. Phytin elimination in soybean protein isolation. J. Amer. Chem. Soc. 79: 633-637.

Sober, H. A., R. W. Hartley, Jr., W. R. Carroll, and E. A. Peterson. 1965. Fractionation of proteins in "The Proteins: Composition, Structure, and Function". V.III., 2nd. ed., Neurath, ed., p. 10-12. Academic Press., N.Y., London.

Spies, J. T., H. S. Bernton, and H. Steven. 1941. The chemistry of allergens IV. An electrophoretic fractionation of the protein-polysacharide fraction, CS-1A, from cottonseed. J. Amer. Chem. Soc. 63: 2163-2169.

Squire, P. G. 1964. A relationship between the molecular weights of macromolecules and their elution volumes based on a model for Sephadex gel filtration. Arch. Biochem. and Biophys. 107: 471-478.

Stefansson, B. R., F. W. Hougen, and R. K. Downey. 1961. Note on the isolation of rape plants with seed oil free from erucic acid. Can. J. Plant Sci. 41: 218-219.

Stefansson, B. R., and F. W. Hougen. 1964. Selection of rape plants (Brassica napus) with seed oil practically free from erucic acid. Can. J. Plant Sci. 44: 359-364.

Tombs, M. P. 1965. An electrophoretic investigation of groundnut protein: the structure of arachins A and B. Biochem. J. 96: 119-133.

Turner, C. W. 1948. Effect of rape seed oil meal on the thyroid of the chick. Poultry Sci. 27: 118-120.

Underhill, E. W. 1965. Biosynthesis of mustard oil glucosides V. Formation of gluconasturtiin form 1- $\gamma$ - phenylbutyrine-$C^{14}$-$N^{15}$ in watercress. Can. J. Biochem. 43: 179-187.

Underhill, E. W., and M. D. Chisholm, and L. R. Wetter. 1962. Biosynthesis of mustard oil glucosides. I. Administration of $C^{14}$- labelled compounds to horseradish, nasturtium, and watercress. Can. J. Biochem. and Biophysiol. 40: 1505-1514.

Van Etten, C. H., Hubbard, J. E., J. M. Mallan, A. K. Smith, and C. W. Blessin. 1959. Amino acid composition of soybean protein fractions. J. Agric. Food Chem. 7: 129-131.

Vaughan, J. G., A. Waite, D. Boulter, and S. Waiters. 1966. Comparative studies of the seed proteins of Brassica campestris, Brassica oleracea, and Brassica nigra. J. Exptl. Bot. 17: 332-343.

Vaughan, J. G., and K. E. Denford. 1968. An acrylamide gel electrophoretic study of the seed proteins of Brassica and Sinapis species, with special reference to their taxonomic value. J. Exptl. Bot. 19: 724-732.

Vaughan, J. G., and A. Waite. 1967a. Comparative electrophoretic studies of the seed proteins of certain species of Brassica and Sinapis.

Vaughan, J. G., and A. Waite. 1967b. Comparative electrophoretic studies of the seed proteins of certain amphidiploid species. J. Exptl. Bot. 18: 100-109.

West, E. S., and W. R. Todd. 1963. Textbook of Biochemistry. p. 295-296, 3rd. ed., The Macmillan Company, New York.

Wetter, L. R. 1965. The chemical composition of rapeseed meal. p. 32-44 in "Rapeseed Meal for Livestock and Poultry -- a Review." Can. Dept. Agric. Pub. 1257, Ottawa, Canada.

Wetter, L. R., and B. M. Craig. 1959. Varietal and environmental effects on rapeseed I. Isothiocyanate and thiooxazolidone content. Can. J. Plant Sci. 39: 395-399.

Wheaton, R. M., and W. C. Bauman. 1953. Non-ionic separations with ion exchange resins. Ann. N.Y. Acad. Sci. V.57, Art. 3, p. 159-176.

Whitaker, J. R. 1963. Determination of molecular weights of proteins by gel filtration on Sephadex. Anal. Chem. 35: 1950-1953.

Wolf, W. J. 1970. Scanning electron microscopy of soybean protein bodies. J. Amer. Oil Chem. Soc. 47: 107-108.

Wolf, W. J., G. E. Babcock, and A. K. Smith. 1962. Purification and stability studies of the 11 S component of soybean proteins. Arch. Biochem. and Biophys. 99: 265-274.

Wolf, W. J., and D. R. Briggs. 1956. Ultracentrifugal investigations of the effect of neutral salts on the extraction of soybean proteins. Arch. Biochem. and Biophys. 63: 40-49.

Wolf, W. J., and D. A. Sly. 1967. Cryoprecipitation of soybean 11 S protein. Cereal Chem. 44: 653-668.

Wolf, W. J., and A. K. Smith. 1961. Food uses and properties of soybean protein II. Physical and Chemical properties of soybean protein. Food Technol. 15: 12-33.